NOTE TO USERS

DISSERTATION

VEGETATION MICROCLIMATE AND PLANT PHYSIOLOGICAL RESPONSES TO CLIMATE CHANGE EXPERIMENTS IN THE HIGH AND LOW ARCTIC

Submitted by

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Graduate Degree Program in Ecology

In partial fulfillment of the requirements

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY PATRICK F. SULLIVAN ENTITLED VEGETATION MICROCLIMATE AND PLANT PHYSIOLOGICAL RESPONSES TO CLIMATE CHANGE EXPERIMENTS IN THE HIGH AND LOW ARCTIC BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Adviser

Department Head/Director

ABSTRACT OF DISSERTATION

VEGETATION MICROCLIMATE AND PLANT PHYSIOLOGICAL RESPONSES TO CLIMATE CHANGE EXPERIMENTS IN THE HIGH AND LOW ARCTIC

Recent and projected changes in the Arctic climate are amplified relative to temperate and tropical regions. Vegetation microclimate and plant physiology were examined along natural and experimental gradients in the High and Low Arctic to assess extant variability and sensitivity to climate change.

Experimental warming increased early season growth rates of *Eriophorum vaginatum*, but rates declined to ambient levels by mid-season. There was no evidence that warming increased annual production, above- or belowground, despite higher rates of early season growth. This observation is consistent with the hypothesis that carbon supply limits the rate of arrival at peak biomass, while nutrients limit the magnitude of annual production.

Experimental increases in winter snow depth led to changes in the physiology of two arctic evergreens that were dependent upon species and ecosystem type. Leaf oxygen isotope and nitrogen analyses revealed qualitatively similar effects of deeper snow on the two species, but the effects differed across ecosystems. Leaf carbon isotope analyses revealed that changes in snowmelt water inputs and leaf nutrient concentrations, which differed across ecosystems, had divergent effects on the gas exchange physiology of the two evergreen species.

Experimental additions of long-wave radiation and water had effects on the vegetation microclimate that were consistent with expectations and other effects that

were unexpected. Infrared lamps warmed the canopy and soils of prostrate dwarf-shrub herb tundra. Greater penetration of supplemental heat to depth was observed when combined with water supplements. Supplemental water did not, however, raise soil water contents. Wind was an important determinant of soil temperatures under ambient conditions and an important modulator of infrared warming.

Leaf gas exchange physiology of *Salix arctica* varied across landscape gradients and in response to supplemental infrared radiation. Soil temperature was an important determinant of stomatal conductance. Variation in stomatal conductance was faithfully recorded in the oxygen isotope ratios of leaf cellulose, when variation in the isotopic composition of source water was removed. Changes in carbon isotope ratios attributable to changes in photosynthetic capacity and those attributable to changes in stomatal conductance were successfully identified when the information contained in carbon and oxygen isotope ratios was combined.

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INTRODUCTION

The global climate is changing at a rate that is unprecedented in modern human experience. Fossil fuel combustion has served as the backbone of industrial development in human society since the 19th century. Consequently, atmospheric CO_2 concentrations have increased dramatically (Keeling and Whorf 2004). Changes in atmospheric chemistry, attributable to human activities such as fossil fuel combustion, are closely correlated with increases in global mean temperatures. Radiatively-active gases absorb long-wave radiation that might otherwise exit the Earth's atmosphere and re-emit a substantial proportion of the absorbed radiation back at the Earth's surface. The close correlation between climate warming and concentrations of radiatively-active gases, combined with our knowledge of their "heat-trapping" capacity, has formed the basis of an international scientific consensus that the recent warming trend is partially a consequence of human activities (IPCC 2001). Conservative projections, which assume atmospheric CO_2 concentrations of ~600 ppm, predict a ~2.5°C increase in global air temperatures and a ~3% increase in global precipitation by 2100 (ACIA 2004).

The Arctic is expected to experience changes in climate that are amplified relative to lower latitudes. As arctic temperatures warm, the duration and effective extent of snow and ice cover are expected to decline though melting and changes in vegetation. Snow and ice tend to reflect, while the terrestrial surface and oceans tend to absorb a greater proportion of incoming short-wave radiation. A relative increase in short-wave absorption and subsequent long-wave emission is expected to amplify increases in

surface air temperatures. A greater proportion of the energy received at the Arctic land surface is re-emitted as sensible, rather than latent heat. Sensible heat emission directly warms the overlying atmosphere. The summer boundary layer in arctic regions is relatively shallow. Therefore, changes in radiative forcing and energy partitioning are expected to elicit surface air temperature responses greater than those expected for lower latitudes.

Changes in the Arctic climate are underway. Observations made during the 20th century revealed rising air temperatures (Chapman and Walsh 1993, Overpeck at al. 1997), increasing precipitation (Dai et al. 1997), receding glaciers (Dyurgerov and Meier 1997), reductions in sea ice extent (Chapman and Walsh 1993), thawing permafrost (Osterkamp and Romanovsky 1996) and rising sea levels (IPCC 2001). The notion that climate change in the Arctic will be amplified relative to lower latitudes is apparently supported by air temperature increases in excess of 0.5°C per decade between 1966 and 1995 at some arctic sites (Chapman and Walsh 1993) and by estimates that precipitation increased by ~8% across the Arctic during the 20th century (ACIA 2004). Conservative estimates predict a ~4°C increase in arctic air temperatures and a ~20% increase in arctic precipitation by 2100 (ACIA 2004).

A wealth of experimental and observational research has enriched our understanding of terrestrial ecosystem structure and function in the Low Arctic. Studies have examined constraints on community composition (e.g., Chapin et al. 1995), aboveground plant growth (e.g., Chapin and Shaver 1996), phenology (e.g., Arft et al. 1999) and ecosystem carbon balance (e.g., Oechel et al. 1993, 1998). These studies examined wet sedge and or tussock tundra, which together account for approximately

10% of the ice-free terrestrial Arctic and hold vast stores of soil C that could be mineralized in a warmer climate (Miller et al. 1983). Soil C in graminoid ecosystems of the Low Arctic is predominantly root-derived (Loya et al. 2004). Methodological limitations have, however, restricted study of the constraints on belowground production. The study described in Chapter I was designed shed light on the controls over root production in tussock tundra.

The majority of ecological research in the Low Arctic has examined wet sedge and tussock tundra. Consequently, our understanding of arctic plant species and their sensitivity to climate change is based upon observations in graminoid ecosystems. Many arctic plant species exist in a wide range of habitats, yet little is known about how individual species vary as a function of habitat. Long-term experiments (e.g., Chapin et al. 1995, Arft et al. 1999), and modeling efforts (Epstein et al. 2000, 2001) have used plant functional types to summarize and predict the response of arctic vegetation to changes in climate, yet little is known about variability within functional types. The study described in Chapter II was designed to test two assumptions implicit in the analysis of data from climate change experiments and in models of arctic vegetation change. First, evergreen shrubs will respond in a qualitatively similar manner to climate change. Second, responses will be qualitatively similar across ecosystem-types.

Ecosystems in the High Arctic have received relatively little scientific attention, despite their importance on an areal basis. Climate change experiments in remote regions of the Arctic and Alpine have frequently used passive greenhouse warming as a method to test the temperature sensitivity of various ecosystem processes (Marion et al. 1997). Greenhouse warming has improved our understanding of the constraints on community

composition (e.g., Chapin et al. 1995), aboveground plant growth (e.g., Chapin and Shaver 1996), phenology (e.g., Arft et al. 1999) and ecosystem carbon balance (e.g., Welker et al. 2000) in arctic ecosystems, yet concerns have been raised about undesirable effects on the diurnal temperature range (Kennedy 1995). The study described in Chapter III was designed to investigate vegetation and soil microclimate effects of infrared warming, as an alternative to passive greenhouse warming in the Arctic.

Leaf photosynthetic capacity is an important regulator of CO_2 exchange, while stomatal conductance is an important determinant of H_2O exchange between vegetation and the atmosphere. Carbon isotope ratios ($\delta^{13}C$) in plant material have been used extensively as an indicator of the balance between photosynthetic capacity and stomatal conductance (Dawson et al. 2002, Ehleringer at al. 2002). Investigators have long suggested that combined use of carbon and oxygen isotope ratios ($\delta^{18}O$) in plant material may be used to differentiate between changes in $\delta^{13}C$ that are driven by photosynthetic capacity and those driven by changes in stomatal conductance (e.g., Farquhar et al. 1989b), yet relatively few studies have examined $\delta^{13}C$ and $\delta^{18}O$ of plant material in the field (Sternberg et al. 1989, Yakir and Israeli 1995, Saurer et al. 1997, Scheidegger et al. 2000, Barbour et al. 2000, Barbour et al. 2002, Cernusak et al. 2005, Sullivan and Welker in review). The study described in Chapter IV was designed to test the ability of the dual isotope ($\delta^{13}C$ and $\delta^{18}O$) approach to reproduce patterns of leaf gas exchange observed in the High Arctic landscape and in response to experimental climate change.

CHAPTER I.

Warming chambers stimulate early season growth of an arctic sedge: results of a minirhizotron field study

Introduction

The interactions between climate change and terrestrial vegetation occur across a variety of spatial and temporal scales (Levin 1992). Because individual plant species differ in their effects on ecosystem structure and function, changes in vegetation composition mediate ecosystem responses to climate change (Eviner and Chapin 2003). For instance, the response of individual species may affect nitrogen cycling (Quested et al. 2003) and litter decomposition (Hobbie 1992, 1996), as well as trace gas (Welker et al. 1993, 1997) and energy (Chapin et al. 2000) exchange with the atmosphere. These changes in ecosystem processes may feed back to amplify or dampen directional climate change. The role of individual plant species in mediating climate change is particularly likely in the Arctic where a few species dominate and their functional characteristics appear to be important controls on ecosystem processes.

Plants may respond to environmental change through changes in the timing, rate or duration of growth (Welker et al. 1997). Understanding details of the intra-annual growth response, particularly in systems where nutrient (Nadelhoffer et al. 1992) and light (Chapin 1983) availability are ephemeral, may provide insights into mechanisms that affect patterns observed at annual time-steps. For instance, an earlier onset or higher rates of early season growth may enhance nutrient uptake and light interception while

these resources are most available. Alternatively, continued growth or delayed senescence may enhance resource capture during the fall when soil thaw is near its annual maximum. These intra-annual growth responses may manifest as changes in annual net primary production, biomass allocation and/or ecosystem carbon balance.

In arctic graminoid tundra, plant species maintain a large proportion of their live biomass belowground (Shaver and Cutler 1979). In contrast with leaf litter, root litter accumulates beneath the soil surface where temperature and moisture conditions are less conducive to decomposition (Nadelhoffer et al. 1992). As a result, soil organic matter in these systems is predominantly root-derived (Loya et al. 2004). Despite the importance of root production as an input to soil organic matter pools, understanding the magnitudes (Lauenroth 2000) and controls (Farrar and Jones 2000) over belowground allocation remains among the greatest challenges in contemporary ecology.

Brouwer (1962) proposed a simple qualitative model to describe biomass partitioning at equilibrium and in response to environmental change. The model predicts that plants will shift allocation toward leaves if aboveground resource availability declines and toward roots if belowground resource availability declines. Because activity levels (i.e., rates of photosynthesis and nutrient absorption) may affect the amount of biomass required to gain carbon or nutrients, Davidson (1969) suggested that the product of root mass and nutrient absorption rate should be proportional to the product of leaf mass and photosynthetic rate. Functional equilibrium has successfully, albeit qualitatively, predicted plant responses to changes in light, nutrient and water supplies (Lambers 1983, Poorter and Nagel 2000).

Warming experiments in tussock tundra have improved our understanding of species and ecosystem responses to simulated change through studies on ecosystem carbon balance, aboveground growth and plant phenology (Chapin and Shaver 1985, Chapin et al. 1995, Chapin and Shaver 1996, Arft et al. 1999, Hobbie et al. 1999, Welker et al. 2000, Bret-Harte et al. 2001, 2002). Warming experiments in tussock tundra have examined root production (Shaver et al. 1986, Chapin and Shaver 1996, Hobbie and Chapin 1998), but the results have been inconsistent. With recent improvements in optic and electronic technology, minirhizotron camera systems have emerged as a useful tool to examine warming effects on root growth *in situ* at high temporal resolution (Hendrick and Pregitzer 1996, Johnson et al. 2001).

In this study, I examined the initial effects of open-top chamber (OTC) warming on growth of *Eriophorum vaginatum* L. ssp. *spissum* (Fern.) Hult. Previous work with small open-top greenhouses in tussock tundra found no effect on soil temperatures (Hobbie and Chapin 1998). I anticipated a similar result and therefore, tested the following question: If warmer air temperatures stimulate carbon assimilation through changes in leaf growth and/or photosynthetic rates, but a lack of soil warming means nutrient absorption rates are unaffected, will air warming lead to an increase in root mass? In addition to testing the predictions of a functional equilibrium, my objectives were as follows: 1) to quantify chamber effects on annual root production over the first three years of warming; 2) to quantify chamber effects on weekly patterns of leaf and root growth, as patterns observed at weekly time-steps may help to explain those observed at annual time-steps.

Materials and Methods

Site and Species Description

The study was performed in arctic Alaska at the International Tundra Experiment (ITEX) moist acidic tussock tundra site near Toolik Lake in the northern foothills of the Brooks Range (68°37'N, 149°36'W; elevation 740 m asl) (Jones et al. 1998, Walker et al. 1999, Welker et al. 2000, Schimel et al. 2004). The study site is on a gentle (ca. 5°) east-facing slope that was deglaciated 10,000 - 60,000 yr B.P. (Hamilton 1986). The soil is a Rustic Histic Aquiturbel with a 20-30 cm organic horizon (Schimel et al. 2004).

Aboveground, *E. vaginatum* produces leaves sequentially (Jonasson and Chapin 1985), such that late season cohorts over winter to resume photosynthesis and growth the following spring (Defoliart et al. 1988, Starr and Oberbauer 2003). Belowground, *E. vaginatum* maintains a simple, largely unbranched, visually distinct annual root system (Chapin et al. 1979, Shaver and Cutler 1979). *E. vaginatum* was selected for intensive study because of its dominance in circum-arctic and boreal systems, its apparent sensitivity to simulated and observed climate change and its suitability for study with minirhizotrons.

Open-top Chamber Treatment

In May 2001, small Sun-Lite HP fiberglass (Solar Components Corporation, Manchester, NH USA) OTC's (Molau and Mølgaard 1996, Marion et al 1997, Hollister and Webber 2000) were installed in a completely randomized experimental design with eight control and six chambered plots. The chamber material is designed to maintain near-natural transmittance of visible wavelengths (~86%) and low transmittance of reradiated infrared wavelengths (<5%) (Molau and Mølgaard 1996). Traditional

hexagonal OTCs were modified by including a seventh panel to increase the area and decrease the strength of the treatment. The modified chambers were 40 cm in height and covered 1.75 m^2 of tundra. In 2001, 2002 and 2003, the chambers were installed immediately following snowmelt and were removed in the final week of August.

Microclimate Measurements

During 2002, inter-tussock soil surface temperatures were monitored at two-hour intervals within and without chambers using Hobo single channel temperature loggers (Onset Computer Corporation, Bourne, MA USA). Ambient air temperature at 1.0 m, photosynthetic photon flux density (PPFD) and precipitation data were obtained from the Campbell CR21x data logger at the Toolik Tussock LTER site, 470 m northwest and 15 m above the ITEX tussock tundra site.

Leaf Length Measurements

Three tillers were tagged near the center of each plot immediately following snowmelt in 2002. Whenever possible, tillers were selected on different tussocks. Leaf cohorts for each tiller were identified based upon time of exsertion such that over-wintering 2001 leaves were considered cohort 1 or 2, depending upon the senescent: green length, while leaves produced during 2002 were identified as cohort 3-5 as they emerged in each tiller. Leaf exsertion was synchronous to within two weeks at the site, and while many tillers did not produce a fifth cohort during the measurement period, all vegetative tillers produced the fourth cohort. Because sequential leaf development ceases when tillers reproduce, all tillers that invested in reproduction during the study were eliminated. Leaf length was measured for each extant cohort on the same dates as minirhizotron root image capture (n = 14), and then averaged at the plot (replicate) level.

To address the potential for growth and senescence of the same leaf between sampling dates, measurements were taken from the collar to the leaf tip, or if senescence had begun, to both the senescence mark and the leaf tip.

Leaf Production Estimates

To convert leaf growth measurements to biomass, the current year's senescent leaf material was collected, dried at 70°C for 24 hours and then weighed, scanned and digitized to determine leaf mass per unit length (LML). The LML estimate within the chambers (0.079 ± 0.006 g/m) was not significantly different than under ambient conditions (0.083 ± 0.004 g/m) ($P = 0.6214$). Consequently, LML was averaged across treatments and the overall mean (0.081 g/m) was used to convert measurements of leaf length to biomass. This LML estimate, which does not include materials resorped and recycled in the subsequent growing season, was 14% lower than that for green leaf material.

Root Measurements

Minirhizotron Installation

E. vaginatum root production was measured using a minirhizotron system (Hendrick and Pregitzer 1996, Johnson et al. 2001). Cellulose acetyl butyrate minirhizotrons were etched with a continuous 0.8 x 1.3 cm grid to assist return of the camera to the same soil volume on successive sampling dates and minirhizotrons were sealed with a rubber stopper to inhibit inflow of soil water. In July 2000, 10 months before inception of the study, minirhizotrons were installed at approximately 45° to the soil surface to approximately 50 cm vertical depth in each of 14 plots. To maintain near

natural thermal conditions within the minirhizotrons, they were filled with removable foam pipe insulation and aboveground portions were painted white to reflect light.

Image Capture and Analysis

Minirhizotron images (~80/minirhizotron) were captured, centered on each grid rectangle, using the BTC-2 minirhizotron camera system and ICAP software (Bartz Technology, Santa Barbara, California) in approximately one-week intervals between snowmelt and late August 2001 and 2002 and in late-season campaigns during the first weeks of October 2001 and September 2003. During this three-year study, approximately 26,000 images were captured and analyzed. The images were analyzed for the current year's root length using the MSU ROOTS software package (Michigan State University, East Lansing, MI), which automatically exports root dimensions to a Microsoft Access database (Microsoft Corporation, Seattle, WA). To eliminate the need for precise camera placement, only those root lengths that appeared within the grid-rectangle were measured and analyzed.

Minirhizotron images were captured weekly and previous images were revisited during analysis to reduce the potential for appearance and disappearance of the same root between sampling dates and to ensure correct root age identification. To assess the extent to which independent late-season samples (analogous to a biomass harvest) underestimate annual root production, late-season 2001 and 2002 images were re-analyzed without revisiting previous images.

Soil Cores

Four soil cores (5.0 cm diameter) were collected to approximately 5 cm beneath the thawed soil interface immediately following minirhizotron image capture on ten dates

during 2002. Cores were taken from the center of arbitrarily selected *E. vaginatum* tussocks, stratified by size, in a representative area outside of the experimental plots. Because of the tussock growth form, root density is highly, but predictably, spatially heterogeneous. Cores were taken from loci of high root density to reduce heterogeneity-induced variance and to better constrain root production estimates. Live *E. vaginatum* roots were separated by cohort from soil cores within five hours of sample collection, serially washed in five de-ionized H_2O baths, dried for 24 hours at 70°C and weighed.

Scaling of Minirhizotron Measurements

Previous studies have converted root length/minirhizotron to root length/m^2 by assuming an image depth of field (Johnson et al. 2001). However, production estimates are highly sensitive to subtle changes in depth of field, which is very difficult to measure and may vary with soil type. Soil cores taken from the center of *E. vaginatum* tussocks were used along with root production estimates and root production as a percentage of total production (Chapin et al. 1988) to calibrate image depth of field for the soils of tussock tundra.

Chapin et al. (1988) sampled from the center of *E. vaginatum* tussocks and reported annual root production of 1568 g/m^2 (~75% of total production) for tussocks in areas of preferential surface water flow (water-tracks) and 288 g/m^2 (~90% of total production) for tussocks in areas outside of the water tracks (non-track). Soil cores taken from the center of tussocks on August 12, 2002 revealed 746.3 ± 91.2 g/m^2 of the current year's root biomass, suggesting that *E. vaginatum* root production at the ITEX site, which lies on a bench beneath a ca. 5° slope, is intermediate between the water-track and non-

track sites examined by Chapin et al. (1988). Differences between soil core estimates and the Chapin et al. (1988) water-track and non-track estimates were analyzed as follows:

$$\text{ITEX} - \text{Non-track/Water-track} - \text{ITEX} = 0.558. \qquad \text{(eq. 1.1)}$$

Chapin et al. (1988) ecosystem-scale *E. vaginatum* aboveground production and tussock-scale estimates of root production as a percent of total production were used to generate *E. vaginatum* root production estimates of 62 g/m^2 for non-track and 239 g/m^2 for water-tracks. These estimates and equation 1 generated an expected annual root production of 125 g/m^2 for the ITEX site. A 3.0 mm image depth of field generated a 2002 ambient minirhizotron root production estimate of 119.2 g/m^2, consistent with expectations. This depth of field estimate has been used in previous studies (Sanders and Brown 1978, Itoh 1985).

Root length/minirhizotron was converted to root length/m^2 using 3.0 mm as the image depth of field and by treating each image volume as indicative of root length density for the corresponding soil surface area to the maximum depth of live root appearance during the course of the study (Johnson et al. 2001). Each grid-rectangle along the upper surface of the minirhizotron was imaged. Consequently, root length/minirhizotron was converted directly to root length/m^2:

$$L_s = (L_m * (d_{max} / I_d)) * (1{,}000{,}000 \text{ mm}^2/\text{m}^2 / (d_{max} / \sin(\alpha)) * I_w), \qquad \text{(eq. 1.2)}$$

where L_s is root length (mm/m^2), L_m is root length (mm/minirhizotron), d_{max} is maximum depth of live root appearance (mm), I_d is image depth of field (mm), α is the angle between the minirhizotron and the ground surface and I_w is image width (mm). Inclement weather necessitated some flexibility in sampling intervals. Consequently, root growth was also examined as a daily rate, calculated as follows:

$$L_{sr} = (L_s \text{ at } t2 - L_s \text{ at } t1) / I, \qquad \text{(eq. 1.3)}$$

where L_{sr} is root length growth rate (mm m^{-2} day^{-1}), t1 and t2 are consecutive sampling dates and I is the period between sampling dates (days).

Sub-samples of the current year's senescent root material were taken from late season soil cores, washed, dried, scanned, digitized for total length and weighed to determine root mass per unit length (RML). On one sampling date, samples were scanned, digitized and weighed prior to and following drying to determine the effect of drying on root length. The RML estimate was then corrected to incorporate a 6% reduction in root length with drying. To avoid excessive destructive sampling within the experimental plots, minirhizotron estimates of root diameter were used as a proxy test for changes in RML. Root diameters within the chambers (0.63 ± 0.03 mm) were not significantly different than under ambient conditions (0.59 ± 0.02 mm) (P = 0.2906). Consequently, the same RML estimate (0.046 g/m) was used to convert chamber and ambient root lengths to biomass. This estimate, which reflects material as it enters the root litter pool, was not significantly different than that for live roots (P = 0.8456).

Inter-annual Variance Dynamics

Equilibration of minirhizotrons with the soil environment (particularly uniformity of the seal between the minirhizotron and the soil) may occur at different rates in different ecosystems. I assumed differential disturbance during minirhizotron installation and that progressive equilibration would manifest as a progressive reduction in variance. To address the potential for equilibration during the course of my study, coefficients of variation were examined for the final sampling date in each year of the project.

Statistical Analyses

The effects of OTCs on soil surface temperatures, leaf and root production rates and root diameters were tested in a repeated-measures framework using the mixed model procedure in SAS 8.2 (SAS Institute, Cary, NC). The restricted maximum likelihood (REML) estimation method was used with random plot effects and an autoregressive lag-one residual correlation structure. To test the effects of OTCs on LML and annual leaf and root production, the general linear model procedure in SAS 8.2 was used to perform analyses of variance (ANOVAs). Correlations between weekly chamber soil surface warming (chamber – ambient) and the magnitude of the response in leaf and root growth rates (chamber – ambient) were examined using the regression procedure in SAS 8.2. When necessary, data were $\log_{10}$ transformed to address increasing variance with increases in the magnitude of the estimates.

Results

Chamber Effects

Under ambient 2002 conditions, PPFD declined while air temperature and precipitation increased with progression of the growing season (Figure 1.1). Between May 21 and August 17, 2002, OTCs warmed daily mean soil surface temperatures by 0.84°C. Immediately following snowmelt, the difference in soil surface temperature between warmed and ambient plots approached a maximum of 1.5°C, but generally declined throughout the period of measurement to approach minimum values around 0.5°C in late August (Figure 1.2). Repeated-measures ANOVA, using hourly mean temperatures on monthly time-steps, identified dependence of the chamber warming

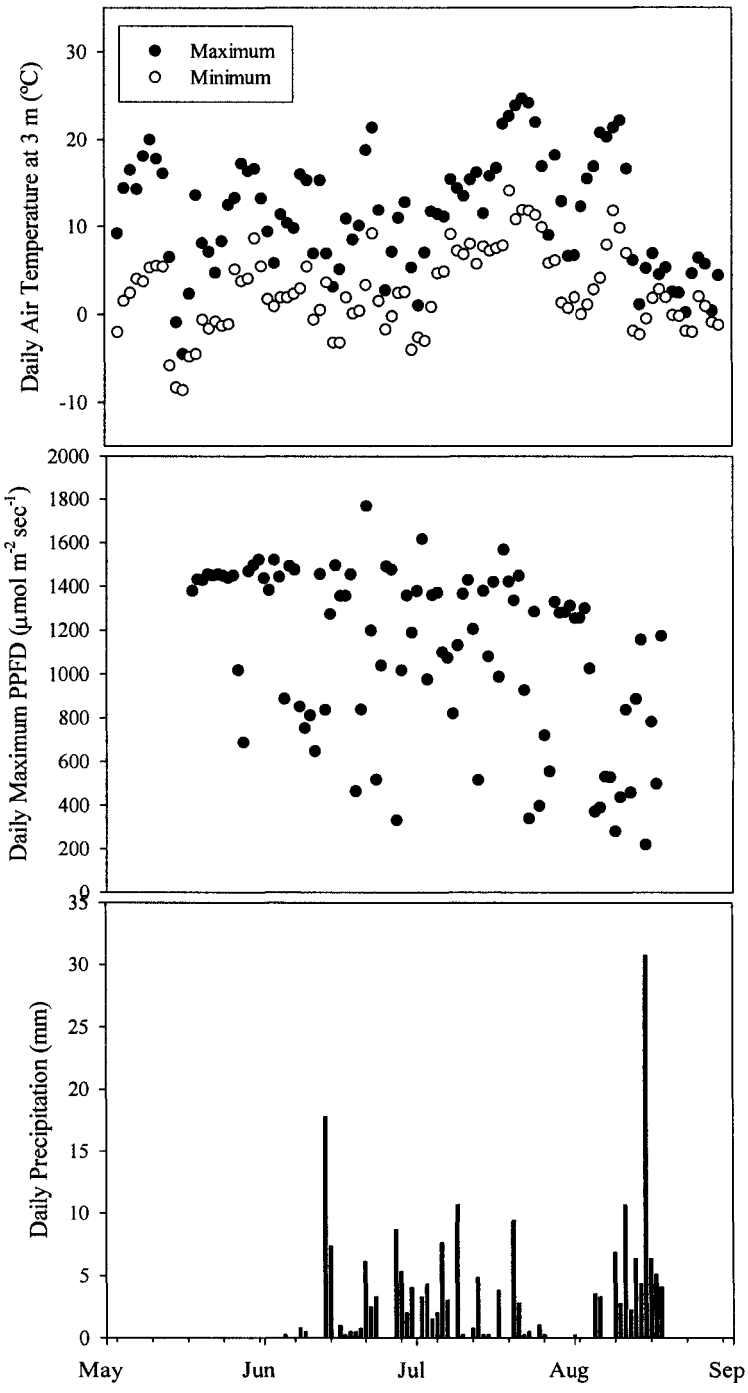

Figure 1.1. Daily maximum and minimum air temperatures (°C), maximum PPFD (µmol m^{-2} sec^{-1}) and precipitation (mm) during the 2002 growing season.

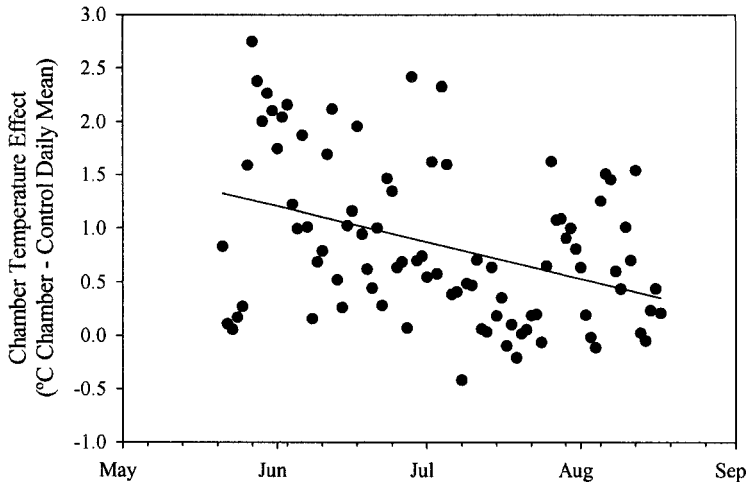

Figure 1.2. Chamber effect on daily mean soil surface temperatures (°C chamber – ambient) observed during the 2002 growing season.

effect upon the hour of observation (F = 4.94, P = <.0001) and dependence of the strength of this interaction on the month of observation (F = 7.05, P = <.0001). Over the course of the growing season, the chamber soil surface warming effect was greatest near the solar maximum and the solar minimum, and least in the early morning and early evening. The magnitudes of these differences were generally greater during the months of May and June.

Leaf Production Response

Under ambient conditions, the rate of leaf growth peaked in early June and mid-July (Figure 1.3). A similar pattern was observed within the chambers, but early season leaf growth rates were higher than those under ambient conditions. Repeated-measures ANOVA identified a significant effect of date, but there was no evidence of an overall chamber effect or of an interaction between the chamber treatment and date (Table 1.1). To further examine the potential for a greater chamber effect during the first half of the growing season, I constructed a linear contrast where the period of measurement was split into seven sampling intervals prior to and seven sampling intervals after July 1. I compared chamber effects on leaf growth rate pair-wise moving away from July 1. For instance, I compared the treatment effect observed during the May 27 to June 3 interval with that observed during the July 28 to August 5 interval. Results of the linear contrast necessitated rejection of the null hypothesis that treatment effects were symmetrical about July 1 and acceptance of the alternative that chambers resulted in higher leaf production rates during the period prior to July 1 (Table 1.1).

I further investigated chamber effects on leaf growth rates at the level of

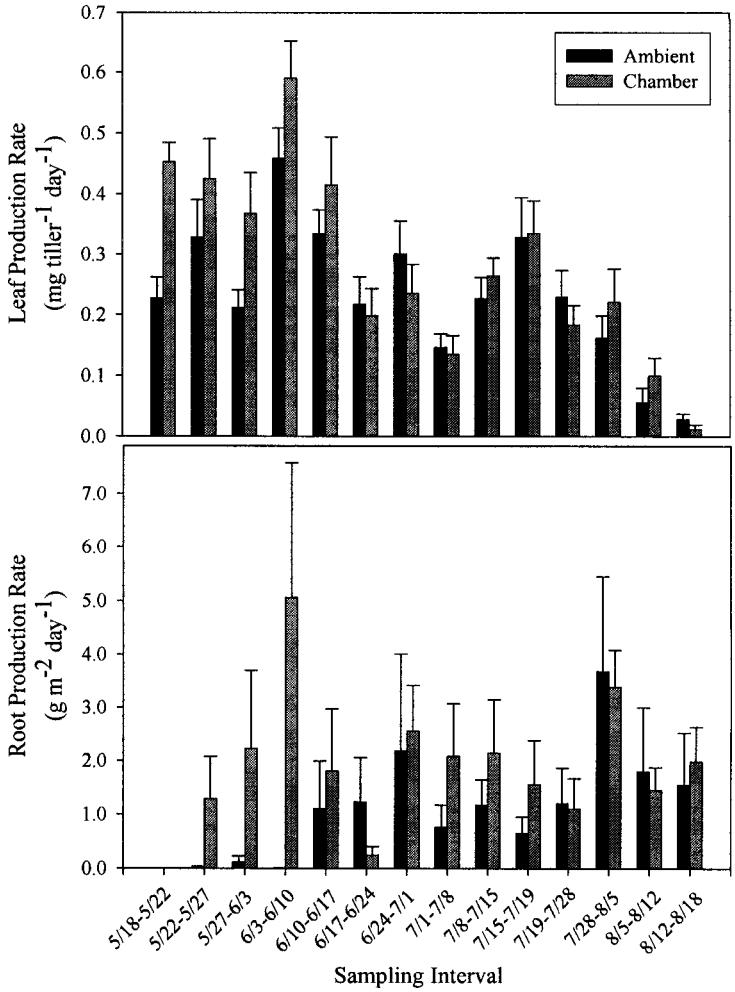

Figure 1.3. Leaf production rate (mg tiller^{-1} day^{-1}) in chamber (n= 6) and ambient plots (n= 8) and root production rate (g m^{-2} day^{-1}) in chamber (n= 5) and ambient plots (n= 7) during the 2002 growing season. Bars are 1.0 SE.

Table 1.1. Results of REML repeated-measures ANOVA's used to assess the effects of open-top chambers on leaf and root production rates over 13 and 12 sampling intervals respectively during the 2002 growing season.

Source	df		*F*		*P*	
	Leaf	Root	Leaf	Root	Leaf	Root
Treatment	1	1	2.46	1.43	0.1431	0.2553
Date	13	12	17.36	4.75	<.0001	<.0001
Treatment x Date	13	12	1.55	2.15	0.1051	0.0172
Linear Contrast	1	.	6.48	.	0.0118	.

individual leaf cohorts. Within chambers, each leaf cohort reached maximum production rates earlier than observed under ambient conditions (Figure 1.4). Consequently, the median date of maximum leaf mass for the third and most productive leaf cohort occurred 16 days earlier, and maximum leaf mass per tiller occurred 20 days before the corresponding maxima under ambient conditions.

Higher early season leaf growth rates within chambers manifest as higher measures of green leaf mass over the first half of the growing season (Figure 1.5). However, following July 1, green leaf mass increased under ambient conditions such that on July 28, there was no longer evidence of a chamber effect.

Root Production Response

I found a significant linear relationship ($r^2 = 0.84$, $P = <.0001$) between estimates of live root biomass derived through sequential coring of *E. vaginatum* tussock centers and live root biomass estimated by minirhizotron image analysis over 11 sampling dates during 2002. The form of the relationship deviated slightly from linearity in the final two sampling campaigns, where there was an increase in the rise of minirhizotron live root biomass estimates per unit rise in soil core live root biomass estimates.

Soil cores taken on August 12, 2002 from the center of tussocks revealed 746.3 ± 91.2 g/m^2 of the current year's root biomass. As tussock centers are loci of high root production, I used this estimate as an upper-bound to screen minirhizotrons for anomalous root production estimates. This criterion resulted in the elimination of one chamber and one ambient minirhizotron from further analyses. Subtle inter-annual differences in late season variance (2003>2001>2002) suggested that progressive equilibration of minirhizotrons with the soil environment was not active during the course

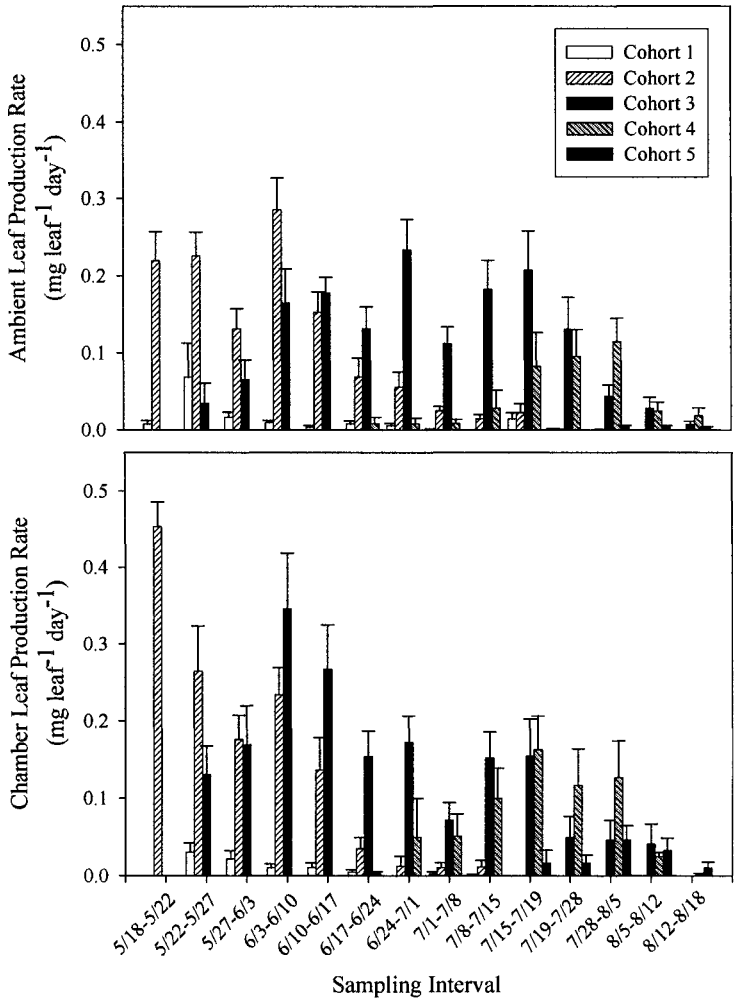

Figure 1.4. Leaf production rate (mg tiller^{-1} day^{-1}) by leaf cohort in plots with (n= 6) and without (n= 8) chambers during the 2002 growing season. Bars are 1.0 SE.

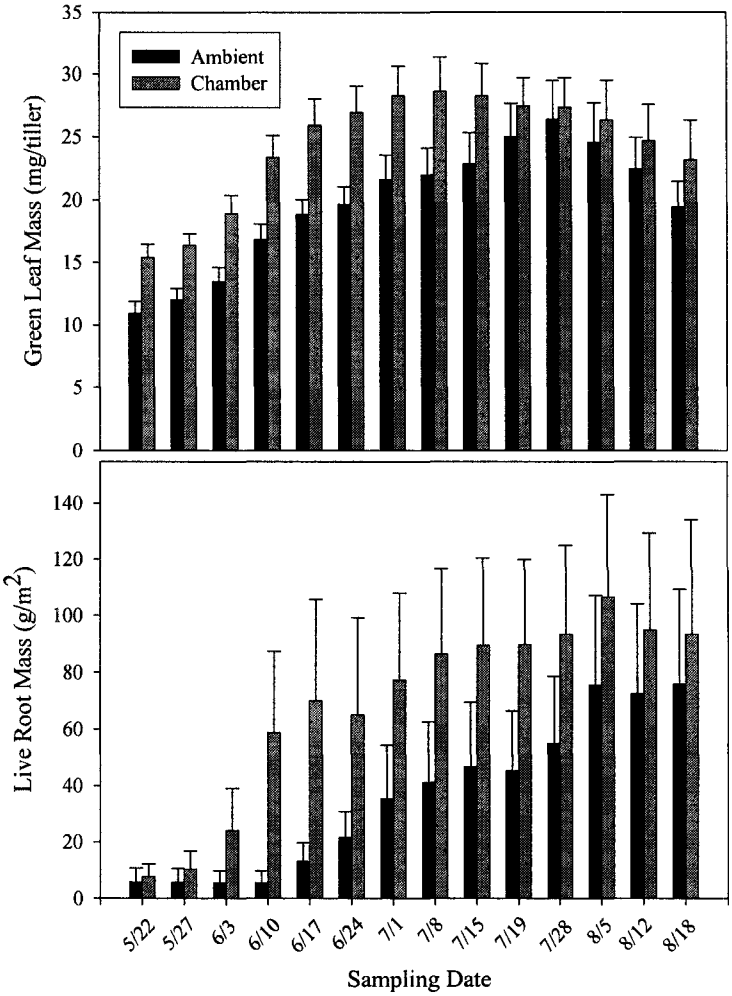

Figure 1.5. Live leaf mass (mg/tiller) in chamber (n= 6) and ambient plots (n= 8) and live root mass (g/m^2) in chamber (n= 5) and ambient plots (n= 7) during the 2002 growing season. Bars are 1.0 SE.

of the study.

Under ambient 2002 conditions, the rate of root growth peaked around the first of July and again near the first of August. I found a similar pattern within the chambers, but the first peak occurred approximately 20 days earlier. Growing season root growth patterns and the timing of the treatment response were similar during 2001, but the magnitude of the treatment response was muted (data not shown). Repeated-measures ANOVA using 2002 root growth rates identified a significant effect of date and dependence of the treatment effect on date, but no significant overall effect of chamber warming (Table 1.1).

As observed aboveground, higher rates of early season root growth within the chambers led to higher measures of live root mass over the first two-thirds of the growing season (Figure 1.5). Late-season 2001 images revealed limited root growth (<10 g/m^2) between August 15 and October 12 and no evidence of a difference in late season growth between ambient and chamber plots ($P = 0.7332$). There was a non-significant trend toward higher root production within the chambers in 2001 (+17%, $P = 0.7746$) and 2002 (+38%, $P = 0.1979$).

Magnitude of Chamber Warming and the Magnitude of Response

There was a weak positive correlation between the magnitude of chamber soil surface warming and the magnitude of the leaf growth rate response ($r^2 = 0.29$, $P = 0.0570$) and no apparent relationship between the magnitude of chamber warming and the magnitude of the root growth rate response ($r^2 = 0.05$, $P = 0.4737$) (Figure 1.6).

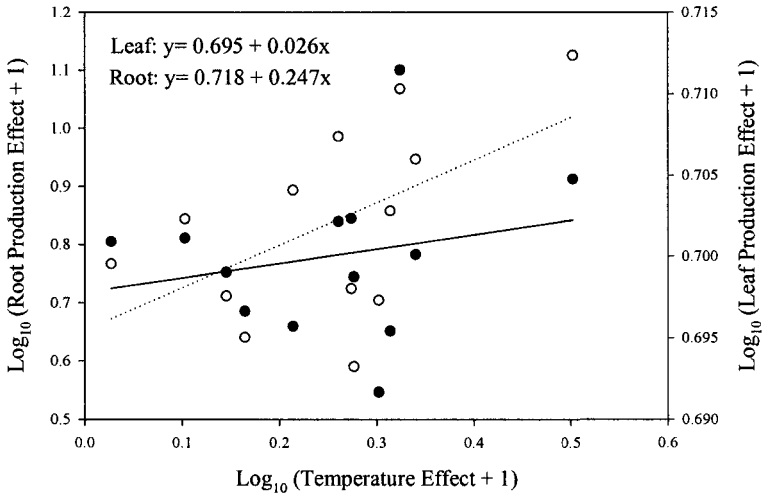

Figure 1.6. Linear regressions of the chamber soil surface warming effect (chamber-ambient) on the response of leaf and root production rates (chamber-ambient) over 13 sampling intervals during the 2002 growing season.

Discussion

Chamber Effects

Observations suggest that surface air temperatures in northwestern North America have increased by approximately 0.5°C per decade since 1966, with the greatest increases occurring during winter and spring (Serreze et al. 2000). While winter conditions were not manipulated in this study, the seasonality (greatest early in the growing season) and the magnitude of OTC warming were consistent with what might be expected in the early decades of the 21st century.

Previous work that characterized the OTC microclimate at the ITEX site found no effect on soil temperatures at 5 cm (A. Parsons unpub. data). This observation is consistent with the results obtained by Hobbie and Chapin (1998) using small open-top greenhouses that treated 1.0 m^2 of tussock tundra.

Leaf Production Response

Early season leaf production rates were higher, maximum rates in each leaf cohort occurred earlier and peak biomass was observed 20 days earlier within the chambers. Tillers within the chambers had significantly higher live leaf biomass throughout the first half of the growing season. However, by late July, ambient and chamber live leaf biomass converged. This convergence arose through the onset of senescence within the chambers and continued production under ambient conditions. There was no evidence of a chamber effect on annual leaf production.

As PPFD peaks soon after snowmelt in arctic ecosystems (Chapin 1983), higher rates of early season leaf growth and an earlier arrival at peak leaf biomass within chambers may have increased light interception over ambient conditions. Tillers within

the chambers maintained an average of 19.7% more green leaf length over 14 sampling dates during 2002. Thus, despite chamber PPFD reductions (<10%) (A. Parsons unpub. data), plants within the chambers likely assimilated more carbon. These observations seem to confirm the hypothesis presented by Shaver et al. (1986) that carbon supply, while it does not limit annual leaf production, does indeed affect the rate at which peak biomass is reached.

Root Production Response

I found a strong linear relationship between minirhizotron live root mass estimates and those obtained through sequential coring of tussock centers, but the relationship deviated from linearity in the final two sampling dates. Failure to identify recently senesced roots in my late season soil cores probably created the observed trend. This result confirms the utility of minirhizotrons in monitoring seasonal root growth patterns and highlights the value of digital imaging as a method to track the fate of individual roots through time. In late season images, when questions arose about the age of individual roots, previous images could be revisited to ensure correct identification.

Earlier studies have reported root production per unit minirhizotron viewing area. Although this approach does not address subtle differences in tube angle, cautious qualitative comparisons can be made across ecosystems. Throughout the 2002 period of measurement to a depth of 40 cm, *E. vaginatum* roots were produced at an average rate of 0.0137 ± 0.0057 mm cm^{-2} d^{-1} under ambient conditions. This rate is lower than that reported by Ruess et al. (2003) (0.023 mm cm^{-2} d^{-1}) over the same depth distribution for floodplain black spruce forests of interior Alaska. Ruess et al. (2003) reported rates of root production on an ecosystem-scale, where *Picea mariana* accounted for

approximately half of aboveground vascular plant production. If *P. mariana* accounted for a similar proportion of root production, the rates would be comparable, if not lower, than those observed for *E. vaginatum*. This comparison highlights the importance of *E. vaginatum* as a vector for carbon transfer belowground.

Early season 2002 root growth rates were higher, maximum growth rates occurred earlier and significantly greater live root biomass was maintained within the chambers over the first two-thirds of the growing season. As observed aboveground, the magnitude of the live biomass difference declined late in the growing season. This convergence probably arose because root production declined and rates of root senescence increased earlier within the chambers.

In tussock tundra, snowmelt is a period of relatively high nitrogen availability, while mid-summer is generally a period of net nitrogen immobilization (Nadelhoffer et al. 1992). Within the chambers, maintenance of greater live root length during the early season period of relatively high nitrogen availability may have increased uptake-efficiency (uptake/available), providing some of the resources necessary to support an increase in annual root production.

There was a non-significant trend toward higher annual root production within the chambers. This result highlights the difficulty of constraining annual root production estimates in spatially heterogeneous ecosystems. In this study, most minirhizotrons intersected tussock root systems to some extent, but a few monitored inter-tussock soils, where *E. vaginatum* root production approaches zero. Although the observed variation in minirhizotron root production estimates (ambient C.V. = 107%, n = 7) could reflect under replication, comparison with a parallel study at a different tussock tundra site, where

three minirhizotrons were installed in each of four blocks (ambient C.V. = 86%, n = 4) (P. Sullivan et al. unpub. data), suggests the variation observed is an inherent feature of tussock tundra. I suggest that future minirhizotron studies in tussock tundra pair one tussock and one inter-tussock minirhizotron, obtain an estimate of tussock rooting area per unit ground surface area and then weight root production estimates appropriately at the plot level.

Although the results of my study alone are insufficient to conclude that short-term climate warming will lead to a change in allocation and to an increase in *E. vaginatum* root production, they are consistent with the predictions of a functional equilibrium, and when combined with similar laboratory results (Kummerow et al. 1980, Kummerow and Ellis 1984), they suggest that short-term air warming may lead to increases in both allocation to roots and annual root production. An increase in *E. vaginatum* root production may affect competitive interactions, stability of soil carbon pools, export of both labile and recalcitrant DOC fractions to aquatic ecosystems, and may partially explain increases in ecosystem respiration that have been observed with OTC warming at the ITEX site (Jones et al. 1998).

Annual root production was underestimated by an average of 35% under ambient conditions and by 60% within chambers when late season 2001 and 2002 images were analyzed without referencing earlier images. This result suggests that independent late season samples are an unreliable method of estimating annual *E. vaginatum* root production and may misrepresent treatment differences. Differences in the underestimates of chamber and ambient root production are probably a consequence of earlier initiation of root production within the chambers. By June 10, plants within the

chambers had produced 32% of their annual total, while plants under ambient conditions had produced only 6%. The difference (26%) corresponds with the difference in the degree of underestimation observed with late season independent sampling. The correspondence between these two figures suggests that chamber air and soil surface warming did not affect root longevity.

Magnitude of Chamber Warming and the Magnitude of Response

There was a weak positive correlation between the magnitude of chamber soil surface warming and the leaf growth rate response, while there was no apparent relationship between the magnitude of warming and the root growth rate response. Aboveground, the weak positive relationship suggests the leaf growth response was partially a quantitative response to increasing temperature and was partially the result of other factors. An increase in early season nutrient uptake and high early season temperature sensitivity may have also contributed to the leaf growth response. Belowground, the lack of a relationship is consistent with the lack of a chamber effect on soil temperatures and suggests the observed root growth response was mediated by changes aboveground.

The results of my study and those of several other passive warming experiments in tussock tundra (Shaver at el. 1986, Chapin et al. 1995, Bret-Harte et al. 2001) suggest that *E. vaginatum* will not decline under climate warming. However, this conclusion appears inconsistent with the experimental (Hobbie and Chapin 1998, Hobbie et al. 1999) and ambient observational results (Chapin et al. 1995) of other studies. Inconsistent reports of the *E. vaginatum* response to warming, many of which come from different time periods at the same site, may reflect differences in the ambient climate. Long-term

periodic monitoring of *E. vaginatum* abundance at a range of sites may be necessary to elucidate the species' trajectory in a changing climate.

CHAPTER II.

Leaf isotopic composition ($\delta^{18}O$ and $\delta^{13}C$) reveals species and ecosystem dependence in the response of two arctic evergreens to deeper snow

Introduction

In the Low Arctic, subtle topographic variation and permafrost give rise to a wide range of soil moisture regimes. In poorly drained lowland wet meadows, gravimetric soil moisture often exceeds 600%, while in dry ridge top communities, moisture levels can be less than 20% (Walker and Walker 1996). Soil moisture has been identified as one of the most important determinants of plant community composition (Walker et al. 1994) and ecosystem carbon exchange (Oechel et al. 1998) in arctic landscapes, but plant physiological responses to changes in moisture have received limited attention. Several lines of evidence suggest that plant physiology may respond to change despite the relatively high soil moisture levels that characterize much of the Low Arctic. Johnson and Caldwell (1975, 1976) reported that photosynthesis in species from moist sites declined to a greater extent with increasing moisture stress than more widely distributed low arctic species. In the High Arctic, Dawson and Bliss (1989) found that female clones of *Salix arctica*, which dominate in wet habitats, do not exhibit the adaptations to moisture stress found in male clones, which dominate in dry habitats. These inter- and intra-specific observations suggest that arctic plants in wet habitats may be just as responsive to changes in moisture as those in dry habitats.

Stable isotope ratios of carbon and oxygen in plant material can be used to infer physiological responses to changes in soil moisture (Saurer et al. 1997, Scheidegger et al. 2000). Leaf $\delta^{13}C$ is linearly related to the ratio of intercellular (c_i) to ambient atmospheric (c_a) CO_2 concentrations (c_i/c_a) (Farquhar et al. 1982, Farquhar et al. 1989). Generally, c_i and soil water availability are positively related in water-limited systems (Smedley et al. 1991, Saurer et al. 1997). The information in leaf $\delta^{13}C$ is somewhat limited, however, as changes in c_i may arise through changes in rates of either photosynthesis or stomatal conductance (g_s) (Farquhar et al. 1989). Within most wild terrestrial plant species, a positive correlation exists between net light-saturated photosynthetic rates (A_{max}) and leaf N, although the strength and the slope of the correlation may vary (Reich et al. 1998). Consequently, investigators have used changes in leaf N to distinguish between leaf $\delta^{13}C$ changes that are driven by changes in g_s and those that arise through changes in A_{max} (e.g., Welker et al. 1993).

Oxygen isotopes in soil water are not fractionated during uptake by roots. Thus, oxygen isotope ratios in stem water directly reflect those of source water (Ehleringer and Dawson 1992). Leaf dry matter $\delta^{18}O$ differs from that of stem water, but carries some of the same water source information. Water in the leaves is enriched relative to source water. Leaf water enrichment is positively related with the difference between source water and atmospheric water vapor $\delta^{18}O$, negatively related with transpiration rate (E) and positively related with the vapor pressure difference (VPD) (Farquhar et al. 1998). When leaf water reaches the chloroplasts, carbonic anhydrase catalyzes the near complete exchange of $^{18}O/^{16}O$ with CO_2. Further enrichment occurs upon incorporation. Cellulose, for instance, is enriched by approximately 27‰ relative to water at the site of

synthesis (Sternberg 1989). Barbour and Farquhar (2000) analyzed cotton leaves grown under controlled conditions and found that enrichment of leaf dry matter above leaf water was closely correlated with that of cellulose, with an offset of -7.5‰. The use of leaf dry matter $\delta^{18}O$ to examine changes in plant water sources has the advantages of a time-integrated measurement (Yakir 1998), but carries a potentially confounding enriched leaf water signal that must be borne in mind.

The $\delta^{18}O$ signature of meteoric water varies with temperature, altitude, latitude, continentality, and precipitation intensity (Dansgaard 1964, Rozanski et al. 1982, Rozanski, et al. 1992, 1993, Welker 2000). In the Arctic, highly seasonal air temperatures give rise to a $\delta^{18}O$ pattern in precipitation that is distinctly seasonal, with winter precipitation that is highly depleted relative to summer rain (Rozanski et al. 1993, Cooper et al. 1993, 1996). Consequently, plants that primarily use snowmelt water may have leaf dry matter that is depleted in ^{18}O relative to plants that use rainwater.

In this study, I selected two circum-arctic/boreal evergreen species (*Vaccinium vitis-idaea* L. and *Ledum palustre* L.) and used leaf $\delta^{18}O$, $\delta^{13}C$ and N to examine their physiological response to nine years of increased winter snow in two very different arctic ecosystems: moist tussock tundra and dry heath tundra. I asked two primary questions: 1. Will the evergreen shrub functional type predict the response of individual evergreen shrub species to increased snow depth (i.e., will the two species respond in a qualitatively similar manner)? 2. Will the response of these two arctic evergreens to increased snow depth depend upon ecosystem type? These questions have important implications for models of arctic vegetation change and the analysis and interpretation of data from field experiments that simulate climate change.

Materials and Methods

Site and Treatment Description

The study was performed in arctic Alaska near Toolik Lake in the northern foothills of the Brooks Range (68°37'N, 149°36'W; elevation 740 m asl) (Jones et al. 1998, Walker et al. 1999, Welker et al. 2000, Schimel et al. 2004). In late-summer 1994, two 60 m long, 2.8 m high collapsible lathe snowfences were established perpendicular to prevailing winter winds. One fence was established in dry heath tundra and the other in moist tussock tundra (Walker et al. 1994). In both ecosystems, the fences create leeward drifts approximately 60 m long, with a maximum depth approximately even with the fence height (Walker et al. 1999). Snow accumulates behind the fences earlier in the fall and, in the deepest part of the drift, snow persists for approximately three weeks longer than in areas of ambient snow depth (Fahnestock et al. 2000). Treatment areas were divided into four 15-20 m zones based upon snow depth: deep (2-3 m), intermediate (0.5-2 m), ambient (~0.5 m), and reduced (<0.5). In this study, samples were collected near the center of the intermediate zones and in areas of ambient snow depth adjacent to the fences. To prevent wind and boundary layer artifacts during the growing season, the fences were laid flat each year in mid-May and re-established in late August.

The moist tussock and dry heath sites are acidic tundra. They were deglaciated 10,000 - 60,000 yr B.P. (Hamilton 1986). The moist tussock site lies on a gentle (ca. 5°) east-facing slope. The soil is a Rustic Histic Aquiturbel with a 20-30 cm organic horizon (Schimel et al. 2004). The dry heath site, which lies approximately 500 m from the moist tussock tundra site, is on the crest of a slightly crowned moraine. The soil is a frigid typic Eutrocryept with a 5 cm organic horizon (Schimel et al. 2004).

Species Descriptions

Two evergreen dwarf shrubs exist in both ecosystems (nomenclature follows Hultén 1968). *Vaccinium vitis-idaea* accounts for 9-10% of vegetation cover at the moist tussock tundra site and 3-5% at the dry heath site. *Ledum palustre* ssp. *decumbens* forms 7-10% of the vegetation cover at the moist tussock tundra site and exists as a minor component of the dry heath community (<1%). While *V. vitis-idaea* inhabits common microtopographic positions of both the dry heath and moist tussock tundra sites, *L. palustre* is generally confined to surface depressions at the dry heath site.

Sample Collection, Processing, and Analysis

Snow cores were collected from the experimental drifts in March of 1995 and 1997, sealed in Ziploc bags, melted in the laboratory, transferred to 125 mL Nalgene bottles and then frozen until analysis. During the 1995 and 1997 growing seasons, samples were collected from individual precipitation events using a 0.5 L Nalgene collection vessel and 20 cm diameter funnel placed at ground level. To reduce the potential for evaporative enrichment, samples were recovered immediately following the cessation of precipitation and frozen until analysis.

On September 13, 2003, a 30 m tape was laid parallel with the snowfence near the center of the intermediate zone in both ecosystems. Individuals of each species (n=5) were selected at random along the tape, and collected all green leaves. Leaf material was collected in the same manner in areas of ambient winter snow adjacent to each snowfence. Leaf life spans of between two and four years have been reported for both *L. palustre* (Shaver 1981) and *V. vitis-idaea* (Kudo et al. 1999), depending upon site fertility and growing season length respectively. Therefore, results are not specific to conditions

during 2003, but integrate over several years of the experiment. Leaf material was oven-dried at 60˚C for 48 hours and ground with a mortar and pestle in the presence of liquid nitrogen.

To measure stable oxygen isotope ratios in winter and summer precipitation, 0.2 mL of each sample was transferred to a 1.0 mL glass vial, aspirated with 10% CO_2 and 90% He in a glove bag and equilibrated for 10 hours at 40ºC. The isotopic composition of CO_2 in the head-space was measured using a multi-prep sampler interfaced with a dual inlet VG-Optima stable isotope mass spectrometer (Micromass UK Ltd., Manchester, Lancashire, UK) (Epstein and Mayeda 1953). To control accuracy and repeatability, samples of known isotopic composition were included every five samples. The average standard deviation of these embedded standards was 0.10‰. Oxygen isotope ratios are reported relative to the Vienna Standard Mean Ocean Water (VSMOW) standard as follows:

$$\delta = (R_{sample} / R_{standard}^{-1}) * 1000, \qquad \text{(eq. 2.1)}$$

where δ is the isotope ratio of the sample in per mil (‰) and R_{sample} and $R_{standard}$ are the isotope ratios of the sample and standard respectively.

Nitrogen concentrations, along with stable carbon isotope ratios in leaf material were measured using a Carlo Erba elemental analyzer (Thermo Electron Corporation, Milan, Italy) interfaced with an Isochrom stable isotope ratio mass spectrometer (Micromass UK Ltd., Manchester, Lancashire, UK). Stable oxygen isotope ratios in leaf material were measured using a EuroVector Pyrolysis unit (EuroVector, Milan, Italy) interfaced with a VG-Optima stable isotope ratio mass spectrometer (Micromass UK Ltd., Manchester, Lancashire, UK) operated in continuous flow mode. To control

accuracy and repeatability, one vacuum oil standard was included every 12 samples and two cellulose standards were included every six samples in carbon and oxygen analyses, respectively. Leaf carbon and oxygen isotope ratios are reported in δ notation relative to the Pee Dee Belemnite (PDB) and VSMOW standards, respectively. The standard deviation of the embedded vacuum oil standards was 0.14‰, while that of the cellulose standards was 0.31‰.

Statistical Analyses

Data were analyzed using SAS 8.2 (SAS Institute 1999). To test the main effects of ecosystem, species, and treatment, along with all possible two- and three-way interactions, Analysis of Variance (ANOVA) was conducted using SAS Proc GLM. Comparisons of interest were made using Tukey's Honest Significant Difference (HSD). The test for an ecosystem effect must be viewed with some caution, as the "ecosystems" are pseudo-replicated. The correlation between leaf $\delta^{13}C$ and leaf $\delta^{18}O$ was examined for each species through Simple Linear Regression using SAS Proc Reg.

Results

Isotopes in Precipitation

Within the experimental snowdrifts, there was limited variation in $\delta^{18}O$ with depth (<3.0‰). Averaging across collections in 1995 and 1997, $\delta^{18}O$ of winter snow was -27.5 ± 0.5‰, while that of summer rain was -16.3 ± 0.3‰.

Leaf N

Leaf N was higher in *L. palustre* (18.2 mg/g) than *V. vitis-idaea* (12.0 mg/g), when averaged across ecosystems under ambient conditions ($P<.0001$) (Figure 2.1). There was no evidence that leaf N differed across ecosystems under ambient conditions,

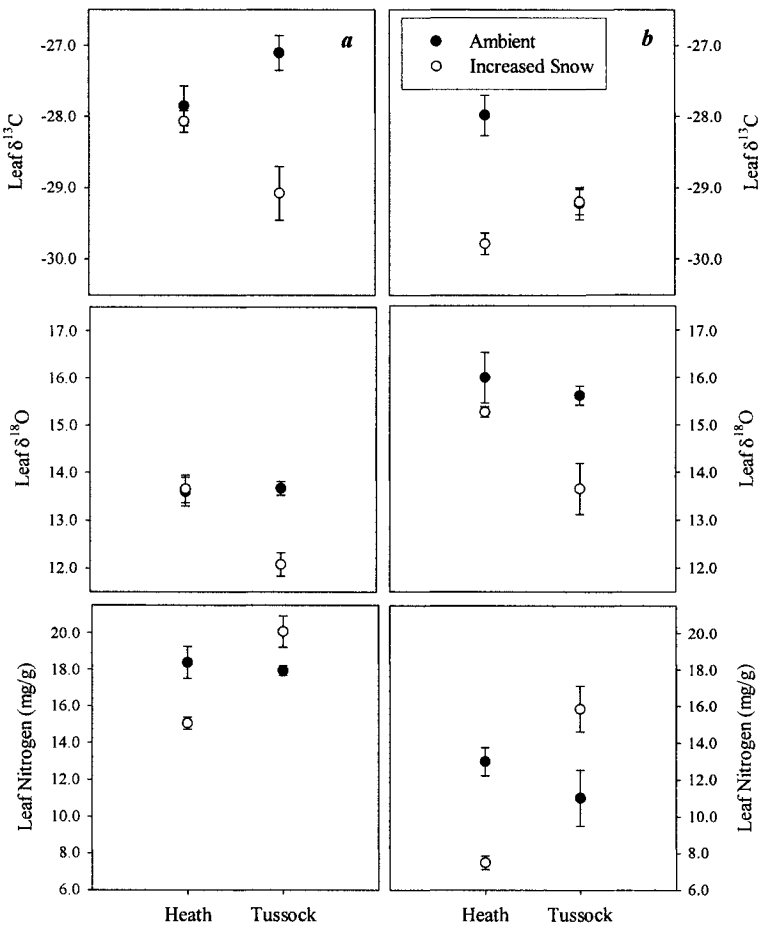

Figure 2.1. Interaction plots depicting variation in *Ledum palustre* (*a*) and *Vaccinium vitis-idaea* (*b*) leaf N, $\delta^{18}O$ and $\delta^{13}C$ across ecosystems and in response to deeper snow. Bars are 1.0 SE.

when averaged across species (P=0.5545). With deeper snow in tussock tundra, there was a significant increase (+3.5 mg/g) in leaf N, when averaged across species (P=0.0023). With deeper snow in dry heath tundra, there were significant declines (-4.4 mg/g) in leaf N, when averaged across species (P=0.0002). Leaf N responses to deeper snow were, therefore, dependent upon ecosystem type (P<.0001) (Table 2.1).

Leaf $\delta^{18}O$

Leaf $\delta^{18}O$ was higher in *V. vitis-idaea* (15.8‰) than *L. palustre* (13.6‰), when averaged across ecosystems under ambient conditions (P<.0001). Leaf $\delta^{18}O$ was lower in tussock tundra (13.8‰) than dry heath tundra (14.6‰), when averaged across species and treatments. With deeper snow in tussock tundra, was a significant decline (-1.8‰) in leaf $\delta^{18}O$, when averaged across species (P<.0001). With deeper snow in dry heath tundra, there was no evidence of a change in leaf $\delta^{18}O$, when averaged across species (P=0.7645). Thus, leaf $\delta^{18}O$ responses to deeper snow were dependent upon ecosystem type (P=0.0054), as observed for leaf N.

Leaf $\delta^{13}C$

Leaf $\delta^{13}C$ was more enriched in *L. palustre* (-27.5‰) than *V. vitis-idaea* (-28.6‰), when averaged across ecosystems under ambient conditions (P=0.0004). There was no evidence that leaf $\delta^{13}C$ differed across ecosystems under ambient conditions, when averaged across species (P=0.7508). With deeper snow in tussock tundra, there was a significant decline in leaf $\delta^{13}C$ of *L. palustre* (-2.0‰) (P<.0001), but no evidence of a change in *V. vitis-idaea* leaf $\delta^{13}C$ (P= 0.9999). With deeper snow in dry heath tundra, there was a significant decline in leaf $\delta^{13}C$ of *V. vitis-idaea* (-1.8‰) (P=0.0003), but no evidence of a change in *L. palustre* leaf $\delta^{13}C$ (P= 0.9982). Therefore, the

Table 2.1. Results of a three-way ANOVA used to describe variation in leaf $\delta^{13}C$, $\delta^{18}O$ and N across species, ecosystems and the increased winter snow treatment.

Source of variation	df	Leaf $\delta^{13}C$		Leaf $\delta^{18}O$		Leaf N	
		F	P	F	P	F	P
Species	1	34.38	<.0001	61.64	<.0001	89.95	<.0001
Ecosystem	1	1.71	0.2005	13.41	0.0010	18.64	0.0001
Treatment	1	32.52	<.0001	19.41	0.0001	0.54	0.4665
Species*Ecosystem	1	0.30	0.5870	0.24	0.6273	0.52	0.4755
Species*Treatment	1	0.01	0.9160	1.45	0.2375	0.04	0.8386
Ecosystem*Treatment	1	0.34	0.5638	9.04	0.0054	38.68	<.0001
Species*Ecosystem*Treatment	1	26.42	<.0001	0.18	0.6750	3.71	0.0633

interaction between deeper snow and ecosystem type was, in the case of leaf $\delta^{13}C$, also dependent upon the evergreen species ($P<.0001$).

Simple linear regression of leaf $\delta^{18}O$ on leaf $\delta^{13}C$ for each species revealed a significant positive correlation for *L. palustre* ($r^2= 0.37$, $P= 0.0056$), but a non-significant correlation for *V. vitis-idaea* ($r^2 = 0.16$, $P = 0.1035$) (Figure 2.2).

Discussion

Isotopes in Precipitation

The $\delta^{18}O$ of winter snow was depleted by more than 10‰, compared with that of summer rain, suggesting that even subtle differences in their relative contributions should affect the $\delta^{18}O$ of early summer soil water. The residence time of the snowmelt water signature in the $\delta^{18}O$ of soil water, and the importance of drainage characteristics remain unknown.

Leaf N

I found higher levels of leaf N in tussock tundra, compared with dry heath tundra, when averaging across species and treatments. This result is qualitatively consistent with previous work at these sites (Welker et al. 2005). In both species, leaf N declined with deeper snow in dry heath tundra, but increased with deeper snow in tussock tundra. This result is also generally consistent with previous work at these sites. Over the first five years of treatment, Welker et al. (2005) found significant increases in leaf N with deeper snow for both *L. palustre* and *V. vitis-idaea* in tussock tundra, but no evidence of a change in leaf N with increased snow in dry heath tundra. The increase in leaf N observed with deeper snow in tussock tundra may be explained by declines in nitrogen productivity (*sensu* Ingestad 1979), as a result of the shortened growing season.

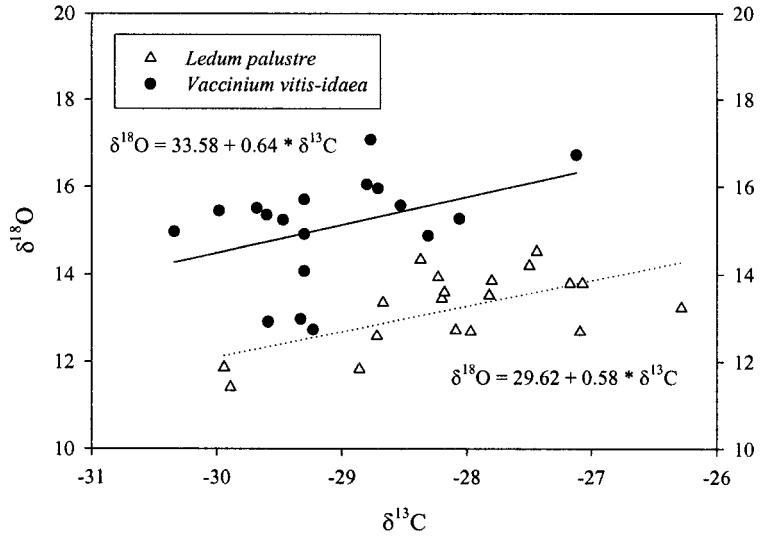

Figure 2.2. Simple linear regressions of leaf $\delta^{18}O$ on leaf $\delta^{13}C$ for *Ledum palustre* and *Vaccinium vitis-idaea* across ecosystems and the winter snow manipulation.

Increases in leaf N with declines in growing season length have been observed along gradients in altitude and latitude (Körner 1989) and along natural snow depth gradients (Kudo et al. 1999). Alternatively, the increase in leaf N with deeper snow in tussock tundra may reflect increases in N availability. Previous work at the tussock tundra site identified increases in both winter and summer net N mineralization with deeper snow (Schimel et al. 2004). After nine years of deeper snow, the significant decline in leaf N observed for both species in dry heath tundra may be attributable to declines in N availability. Further discussion of this hypothesis will follow in the section on leaf $\delta^{18}O$ responses.

Leaf $\delta^{18}O$

With deeper snow in tussock tundra, I found a significant decline in leaf $\delta^{18}O$, but with deeper snow in dry heath tundra, I found no evidence of a change in leaf $\delta^{18}O$. Lower leaf $\delta^{18}O$, in both species, with deeper snow in moist tussock tundra may be explained by reduced leaf water enrichment with deeper snow or by greater use of the relatively depleted snowmelt water source.

Leaf water enrichment is positively related with the difference between source water and atmospheric water vapor $\delta^{18}O$, negatively related with E and positively related with the VPD. Dry heath and moist tussock tundra differ widely in soil water contents, surface energy budgets, vegetation physiognomy and canopy microclimates. These differences are presumed to affect E, may affect canopy VPD and could affect $\delta^{18}O$ of canopy water vapor. However, I found no evidence that leaf $\delta^{18}O$ of either species varied across ecosystems under ambient conditions. Therefore, it seems unlikely that deeper winter snow in tussock tundra increased the difference between source water and

atmospheric water vapor $\delta^{18}O$, increased *E* and or reduced the VPD sufficiently to reduce leaf water enrichment and generate the observed decline in leaf $\delta^{18}O$. In the absence of variation in leaf $\delta^{18}O$ across ecosystems under ambient conditions, I conclude that observed declines in leaf $\delta^{18}O$ with deeper snow in tussock tundra reflect use of the relatively depleted snowmelt water additions during the period of leaf expansion. This conclusion relies on the assumption that ambient dry heath and moist tussock tundra differ to a greater extent in the difference between source water and atmospheric water vapor $\delta^{18}O$, *E* and the VPD than tussock tundra with and without additional winter snow.

The absence of a leaf $\delta^{18}O$ response to increased winter snow in dry heath tundra may be the result of extensive leaf water enrichment following snowmelt. This explanation seems unlikely, as there was no evidence of extensive leaf water enrichment following snow manipulation in tussock tundra and there was no evidence of a difference in leaf $\delta^{18}O$ across the ecosystems under ambient conditions. The absence of a leaf $\delta^{18}O$ response to increased winter snow in dry heath tundra probably reflects the use of relatively enriched rainwater during the period of leaf expansion, rather than water from the experimental snowdrift. I hypothesize that, because the soils of dry heath tundra thaw deeply (>1 m), are highly porous and are low in organic matter (Walker and Walker 1996), snowmelt water from the experimental drift may drain rapidly from the system and may not be used by *L. palustre* or by *V. vitis-idaea* during the period of leaf expansion. These soil characteristics contrast with those of tussock tundra, where the highly organic soils thaw to approximately 50 cm. The recognition that snowmelt water may drain from the system prior to leaf expansion has implications for the observed declines in leaf N with increased snow in dry heath tundra. Increased flushing may leach

both dissolved organic N and winter mineralized N from the system, thereby depressing N availability. Over nine years of treatment, I hypothesize that increased flushing may have exacerbated N limitations to plant production in dry heath tundra.

Leaf $\delta^{13}C - \delta^{18}O$ Correlations

In *L. palustre*, I found a significant positive correlation between leaf $\delta^{13}C$ and $\delta^{18}O$. A positive correlation between leaf $\delta^{13}C$ and $\delta^{18}O$ could be the result of a Péclet effect, as stomatal closure (increasing $\delta^{13}C$) favors back diffusion of evaporatively enriched leaf water (increasing $\delta^{18}O$). I suggest, on the basis of similar leaf $\delta^{18}O$ across ecosystems under ambient conditions, the positive correlation is not driven by a Péclet effect. Instead, the positive correlation between leaf $\delta^{13}C$ and $\delta^{18}O$ could be the result of increasing g_s or declining A_{max} with increasing use of isotopically depleted snowmelt water. Considering that I found the lowest leaf $\delta^{13}C$ and leaf $\delta^{18}O$ under conditions where I found the highest leaf N, declining A_{max} is an unlikely explanation. I suggest that the positive correlation between leaf $\delta^{13}C$ and leaf $\delta^{18}O$ reflects increasing g_s with increasing use of snowmelt water, and that a Péclet effect is secondary. This conclusion is consistent with previous work at high-latitudes (Welker et al. 1995).

Although a similar slope was found in *V. vitis-idaea*, the correlation between leaf $\delta^{13}C$ and leaf $\delta^{18}O$ was not significant, suggesting that some of the variation in leaf $\delta^{13}C$ may not derive from snowmelt water effects on g_s. *V. vitis-idaea* showed its greatest leaf $\delta^{13}C$ response to the snow manipulation in dry heath tundra, where the species showed no change in leaf $\delta^{18}O$ and a decline in leaf N. Under these conditions, I hypothesize that declines in N availability with nine years of snowmelt water flushing reduced leaf N and A_{max} in *V. vitis-idaea*, which led to an increase in c_i and the observed decline in leaf $\delta^{13}C$.

Combining Leaf N and $\delta^{18}O$ to Interpret Changes in $\delta^{13}C$

Response of Ledum palustre in Dry Heath Tundra

I found lower leaf N and no change in leaf $\delta^{13}C$ of *L. palustre* with deeper winter snow. There was no evidence that the species used the experimental snowmelt water during leaf expansion. These results suggest that leaf gas exchange physiology did not respond to the snow manipulation (Figure 2.3a). The absence of a response, despite leaf N levels that were almost 20% lower than observed under ambient conditions, suggests that leaf N does not strongly limit A_{max} in *L. palustre*. This conclusion complements an observation of Chapin and Shaver (1996), that nutrient additions and higher leaf N do not stimulate photosynthetic rates in *L. palustre*.

Response of Ledum palustre in Moist Tussock Tundra

I found higher leaf N and lower $\delta^{13}C$ in *L. palustre* with deeper snow. There was evidence that the species used the experimental snowmelt water during leaf expansion. The observed declines in leaf $\delta^{13}C$ could be attributed to either higher rates of g_s, or lower A_{max}. Given that lower leaf $\delta^{13}C$ was observed in concert with higher leaf N, lower A_{max} is an unlikely explanation. Therefore, I suggest that experimental snowmelt water additions in tussock tundra increased rates of g_s in *L. palustre* (Figure 2.3b). Although increased rates of g_s appear to dominate the leaf $\delta^{13}C$ signature, more subtle increases in A_{max} cannot be ruled out.

Response of Vaccinium vitis-idaea in Dry Heath Tundra

I found lower leaf N and lower leaf $\delta^{13}C$ in *V. vitis-idaea* with deeper snow. There was no evidence that the species used the experimental snowmelt water during leaf expansion. Again, the observed declines in leaf $\delta^{13}C$ could be attributed to either higher

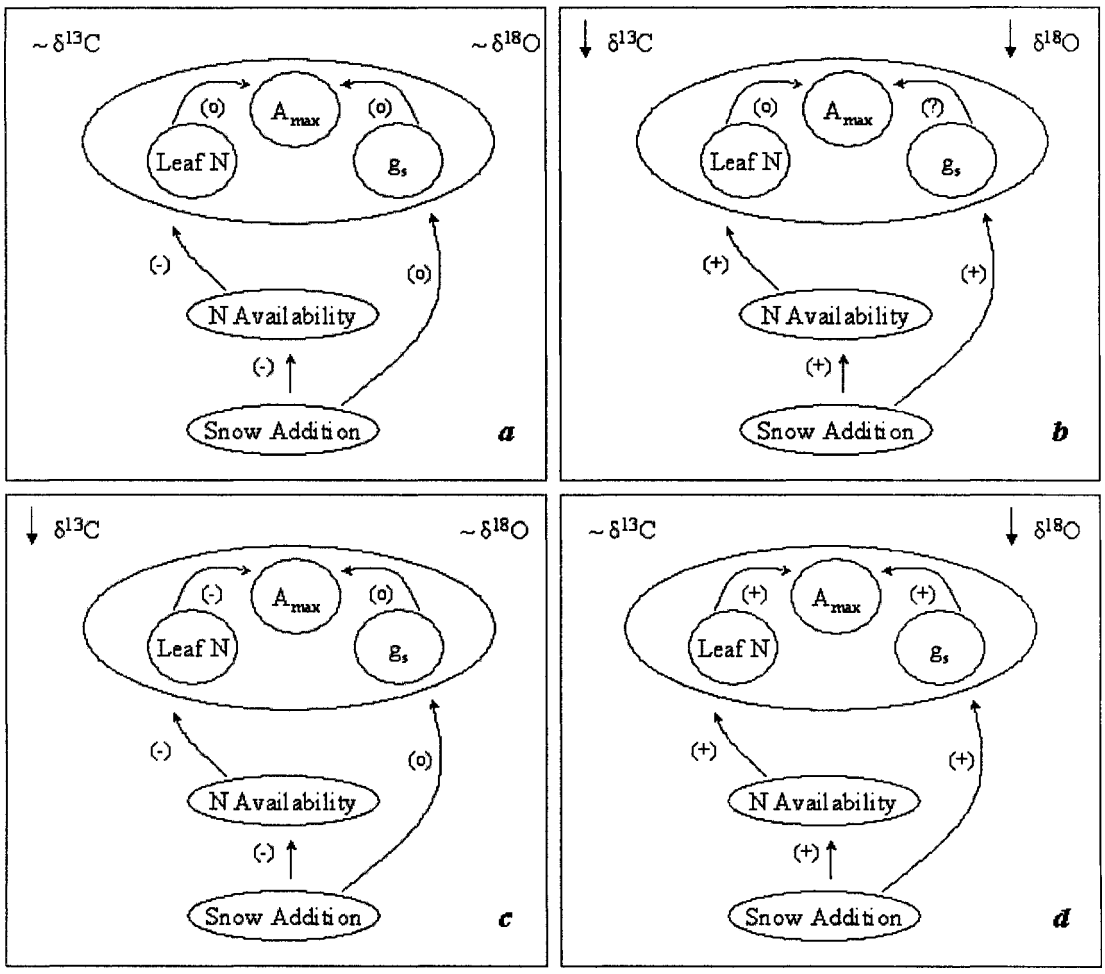

Figure 2.3. Conceptual models depicting hypothesized effects of increased winter snow on soil N availability and leaf physiology of *Ledum palustre* in dry heath (*a*) and tussock tundra (*b*) and *Vaccinium vitis-idaea* in dry heath (*c*) and tussock tundra (*d*). Observed changes in leaf isotopic composition appear in the upper corners (arrows indicate the direction of the response to deeper snow, ~ indicates there was no evidence of a change). Within the models, hypothesized positive (+), negative (-), neutral (o) and unknown (?) influences are indicated.

rates of g_s, or lower A_{max}. As there was no evidence that *V. vitis-idaea* used the experimental snowmelt water, I suggest that lower leaf N reduced A_{max} and led to lower leaf $\delta^{13}C$ (Figure 2.3c). This conclusion implies that A_{max} of *V. vitis-idaea* is more sensitive to changes in leaf N than A_{max} of *L. palustre*. The plausibility of this hypothesis is supported by observations reported in Reich et al. (1998), which demonstrate that the strength of the A_{max} – leaf N correlation varies with species.

Response of Vaccinium vitis-idaea in Moist Tussock Tundra

I found higher leaf N and no change in leaf $\delta^{13}C$ of *V. vitis-idaea* with deeper snow. There was evidence that the species used the experimental snowmelt water during leaf expansion. Building upon my conclusion that A_{max} in *V. vitis-idaea* is sensitive to changes in leaf N and that experimental snowmelt water additions increased rates of *L. palustre* g_s in tussock tundra, I hypothesize that higher *V. vitis-idaea* leaf N increased A_{max}, but experimental snowmelt water additions increased rates of g_s, such that there was no net effect on c_i (Figure 2.3d).

Long-term experiments (e.g., Chapin et al. 1995, Arft et al. 1999), and modeling efforts (Epstein et al. 2000, 2001) have used plant functional types to summarize and predict the response of arctic vegetation to changes in climate. The results of my study complement the conclusion of Epstein et al. (2001), that vegetation aggregation may fail to capture ecosystem properties and processes that are revealed at lower levels of aggregation, and suggest that the evergreen shrub functional type may not be a good predictor of the response of individual evergreen species to changes in climate. Chapin et al. (1996) presented a quasi-objective dendrogram that depicts the relatedness of arctic and boreal plant species at several levels of resolution. In the first step beneath the

functional type, *L. palustre* diverges from the rest of the evergreen shrubs, which remain aggregated. The results of my study support this functional divergence. Future studies that examine the response of arctic evergreens to climate change should verify that *V. vitis-idaea* and the other evergreen shrubs can be aggregated without excessive information loss.

My observation of ecosystem dependence in the response of both *L. palustre* and *V. vitis-idaea* to climate manipulation complements the results presented by Jones et al. (1999), which demonstrate habitat-specificity in the response of another arctic species, *Salix arctica*, to experimental warming. To reproduce the patterns I have observed, a model of vegetation dynamics should recognize differences among evergreen shrubs and the potential for interactions among species and ecosystem types in response to changes in snow depth.

CHAPTER III.

Wind and water modulate experimental energy supplements in a high arctic ecosystem

Introduction

General circulation models predict that arctic regions will experience a warming trend that is amplified relative to temperate and tropical regions (Serreze et al. 2000, ACIA 2004). Long-term records from western Greenland (1873-2001) and more contemporary records from northwestern Greenland (1961-1990) show strong warming trends during summer (Box 2002), consistent with expectations. Further increases in arctic temperatures, on the order of 4-7°C, are expected for the 21st century (ACIA 2004).

Increases in precipitation are expected to coincide with rising temperatures in the 21st century (Serreze et al. 2000, ACIA 2004). Precipitation data for high latitude regions suffer from a sparse station network, with short and/or discontinuous records, and problems associated with undercatch of snow (Serreze et al. 2000). Nevertheless, it is estimated that precipitation across the Arctic increased by approximately 8% during the 20th century. Climate models project that annual arctic precipitation will increase by an additional 20% over the 21st century (ACIA 2004).

High latitude ecosystems are important components of the global climate system because they occupy a substantial proportion of the terrestrial surface, exhibit high spatial and temporal variability in their surface energy budgets and, in some cases, hold large stores of soil carbon (Chapin et al. 2000). In the High Arctic, soil moisture appears to be

the most important determinant of the surface energy balance (Eugster et al. 2000). If changes in climate lead to soil drying, increases in sensible heat flux, which directly feed back to increase air temperatures, may accelerate regional warming. If soil moisture increases with climate change, reductions in sensible heat flux may feedback to dampen regional warming. Because changes in temperature and precipitation are expected to coincide, the net effect of these changes on soil moisture is uncertain. This uncertainty is compounded by a paucity of reliable precipitation and soil moisture data from high latitude weather stations and by the limited capacity of atmospheric models to simulate precipitation (Chapin et al. 2000).

Field manipulations, intended to simulate climate change, have become more common in recent years (Shen and Harte 2000). These studies have provided valuable insights into both the pattern and process of climate change-induced ecosystem change. Climate variables are highly interactive and are not expected to change in isolation (Serreze et al. 2000, IPCC 2001, ACIA 2004). Thus, factorial manipulations of multiple climate variables have helped to isolate underlying mechanisms and provide a more realistic depiction of ecosystem change in the High Arctic (Wookey et al. 1993, Welker et al. 1993, Havstrom et al. 1993, Baddeley et al. 1994, Dormann 2003, Illeris et al. 2003).

This paper describes the design and microclimate effects of an experiment established during June 2003 in prostrate dwarf-shrub, herb tundra (CAVM Team 2003) near Pituffik (Thule), Greenland. Air, canopy and soil temperatures, as well as soil water contents, were examined in response to long-wave radiation and water supplements, applied singularly and in combination. I anticipated that air temperatures would not be

affected by radiation supplements (Saleska et al. 1999), as there are too few molecules of radiatively-active gases between an IR lamp and the plant canopy. I expected that IR lamps would increase both canopy and soil temperatures, but I anticipated reduced soil warming in irrigated plots, as more of the supplemental radiation would be expended in evaporating water, rather than warming the soil (Harte et al. 1995). Harte et al. (1995) and Wan et al. (2002) found soil drying beneath IR lamps. I expected a similar result. Lastly, I anticipated higher soil water contents in plots that received supplemental water.

Materials and Methods

Site Description

The experiment was established in prostrate dwarf-shrub, herb tundra in Pituffik (Thule), Greenland (76° 33'N, 68° 34'W; elevation 180 m asl). Prostrate dwarf-shrub, herb tundra occupies approximately 8% of the ice-free Arctic land surface (CAVM Team 2003). Between 1978 and 2000, mean annual air temperature and precipitation for the site were –11.6°C and 11.6 cm respectively. Over the same period, growing season (June, July and August) air temperatures averaged 3.8°C and approximately 50% of precipitation fell between October and April as snow. Vascular plant cover is approximately 50%. Areas without plant cover are characterized by frost boils, which typically grade from bare soil near the active center to 100% cryptogamic cover at the periphery. The vascular plant community is dominated by three species: the deciduous dwarf-shrub *Salix arctica*, the graminoid *Carex rupestris* and the wintergreen dwarf-shrub *Dryas integrifolia*. The live biomass and litter of these three species account for approximately 70% of vascular plant cover. The soil is a Typic Haploturbel (Soil Survey Staff 1998), with a maximum thaw depth slightly greater than 1 m.

Experimental Design

Three relatively homogenous 70 x 16 m blocks of tundra were selected and five treatments were assigned to 2.0 x 0.8 m plots in a randomized complete block (RCB) design: ambient (A), irrigated (W), infrared level I (T1), infrared level II (T2) and infrared level II x irrigated (T2W). This approach ensured equal distribution of the infrared lamp power requirement across the blocks. Each treatment regime was replicated twice in each block, such that n= 6 at the site-level. At the ecosystem-scale, vascular plants and bare soil/cryptogamic crust each cover 50% of the ground surface. Plots were oriented to span the transition between vascular plants and an individual frost boil, such that each comprised approximately 50%, to facilitate scaling from the plot- to ecosystem-level.

Infrared (IR) lamps (Kalglo Electronics Co. Inc., Bethlehem, PA), 1.6 m in length and 12 cm wide, were suspended 125 cm above the soil surface from rebar tripods installed at the end of each IR plot (Figure 3.1). Testing of the IR lamps prior to field use revealed a non-uniform distribution of the infrared load. A 1.6 m long 5 cm diameter copper pipe was suspended 15 cm beneath the element to distribute the IR flux more evenly over the plots. The spatial distribution of the experimental radiation flux was measured on one date under consistent cloudy conditions using a thermopile probe with a spectral coverage of 0.19 to 6 μm (Oriel Instruments, Stratford, CT). The radiation distribution with and without the copper pipe was measured in several plots. This exercise revealed a 45% reduction in the standard deviation of within-plot radiation levels when the copper pipe was in place. In 2004, the second year of the experiment, the IR treatments were initiated on June 4, the first day that plots were 50% snow-free, and

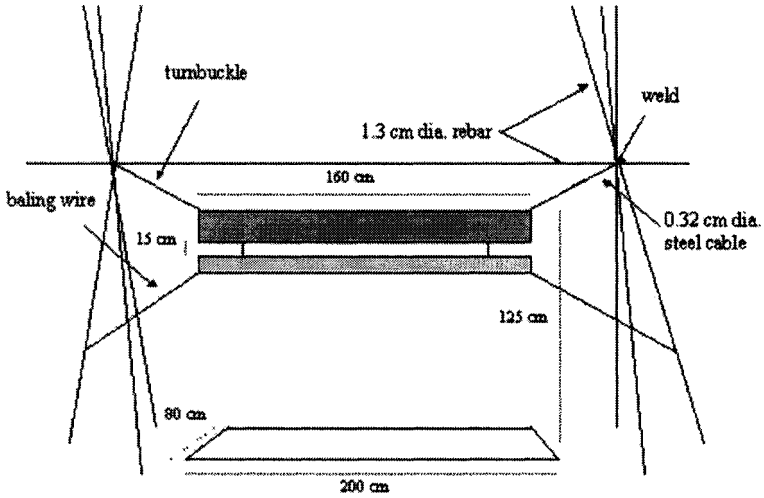

Figure 3.1. Graphical depiction of the support structure used to suspend the IR lamps. Rebar joints were welded and 0.32 cm diameter steel cable were used to withstand winds in excess of 20 m/s.

suspended on August 20, before snowpack development.

Monthly mean precipitation data from 1990 through 2000 were used to design an irrigation treatment that approximately doubles ambient precipitation. During the 10-year record, precipitation during July was approximately twice that recorded during June and August. Consequently, plots were irrigated weekly with 5.0 mm during July and with 2.5 mm during June and August. Irrigation water was passed through a 1μm activated carbon prefilter (General Electric, Co., Fairfield, CT), an activated carbon mixed-bed prefilter and a mixed-bed ultra-pure filter (Barnstead International, Dubuque, IA). To reduce advective soil warming and evaporative loss during irrigation, the water was equilibrated with ambient temperatures for at least 24 hours and applied to plots in the evening.

Microclimate Monitoring

Climate at the study site was monitored by installing a meteorological tower, including sensors for solar radiation, wind speed and precipitation. Solar radiation was measured with a LI200 pyranometer (Licor Biosciences, Lincoln, NE), wind speed at 3 m was measured with a 05103 wind monitor (R.M. Young Co., Traverse City, MI) and precipitation was measured with a 12" heated rain gauge (Met One Instruments. Inc., Grants Pass, OR). Hourly-averaged measurements were logged to a CR23X datalogger (Campbell Scientific, Logan, UT).

Hourly air temperatures at 20 cm and soil temperatures at 5 and 10 cm beneath a closed *D. integrifolia* canopy were measured using Hobo outdoor 4-channel external temperature loggers (Onset Computer Corp., Bourne, MA). In each plot (n=30), temperatures at 2 cm beneath a closed *D. integrifolia* canopy were measured using

Thermochron iButton temperature loggers (Maxim/Dallas Semiconductor Corp., Dallas, TX).

Mid-day *D. integrifolia* canopy temperatures (n=10) were measured in each plot with an infrared temperature probe (Fluke Corporation, Everett, WA). Canopy temperatures were measured under a variety of weather conditions from 5 cm above the canopy, where the probe has a 1.5 cm diameter field of view.

Volumetric soil water content in the upper 12 cm was measured on weekly intervals in each plot using a hand-held HydroSense time domain reflectometer (TDR) probe (Campbell Scientific, Logan, UT). To better resolve the effects of irrigation on temporal patterns of soil water content, eight additional plots were established (4 irrigated and 4 ambient) and, over three days in early August, volumetric soil water content (as described above) and gravimetric soil water content in the upper 5 cm were measured 1 hour, 24, 48 and 72 hours after irrigation with 5.0 mm of water.

Statistical Analyses

To estimate the infrared radiation addition to each plot, spatial trends in the radiation data were fit with a second-order polynomial and universal kriging was used to interpolate the residuals in S-Plus 4.0 (MathSoft Engineering and Education Inc., Cambridge, MA). Variation in canopy temperatures across treatment and weather regimes, variation in radiation inputs and soil temperatures across treatments, weekly variation in ambient volumetric soil water content and responses of volumetric and gravimetric soil water contents to irrigation were examined using analysis of variance (ANOVA) in the general linear model (GLM) procedure of SAS 9.1 (SAS Institute, Cary, NC). Comparisons of interest were made using Tukey's Honest Significant Difference

(HSD). To examine implications of the experimental radiation additions for soil temperatures at 2 cm and to explore statistical relationships between soil warming, soil temperatures and wind speeds, the regression (REG) procedure was used in SAS 9.1. Differences in slope of the regressions relating wind speeds and soil temperatures on hourly time-steps were tested using analysis of covariance (ANCOVA) in the GLM procedure of SAS 9.1.

Results

Radiation additions (means of kriged surfaces) in T1, T2 and T2W plots were 30, 57 and 49 W/m^2 respectively (Figure 3.2). There was no evidence that air temperatures at 20 cm were significantly different than ambient in T1 (P= 0.4733) or T2 (P= 0.1795) plots.

The effects of experimental radiation inputs on canopy temperatures were dependent upon weather (P= 0.0012). There was no evidence of a significant effect of the treatments on canopy temperatures under direct solar radiation, nor under high winds (Figure 3.3). When solar radiation was diffuse (i.e., cloudy conditions), canopy temperatures were significantly higher than ambient in T1 (P= 0.0001), T2 (P= 0.0010) and T2W plots (P< .0001), though not significantly different from one another. Weather affected within-plot variability in canopy temperatures (P<.0001). Within-plot coefficients of variation (CV's) were greatest under direct radiation (13.2%), intermediate under diffuse radiation (10.5%) and least under windy conditions (7.9%).

Simple linear regression of mean plot-level soil temperatures at 2 cm (June 8 – August 19, 2004) on radiation input at the soil surface (July 1, 2004) revealed a

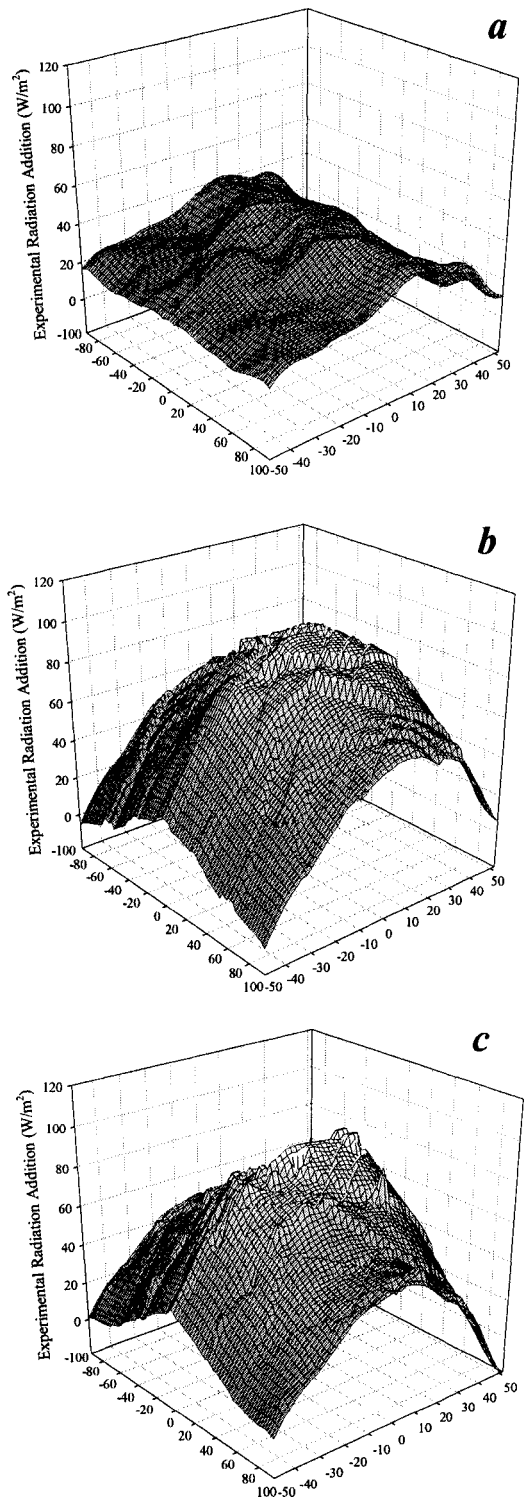

Figure 3.2. Spatial distribution of the experimental radiation flux at the soil surface. Surfaces are means for the T1 (*a*, n= 4), T2 (*b*, n= 4) and T2W (*c*, n=6) treatments.

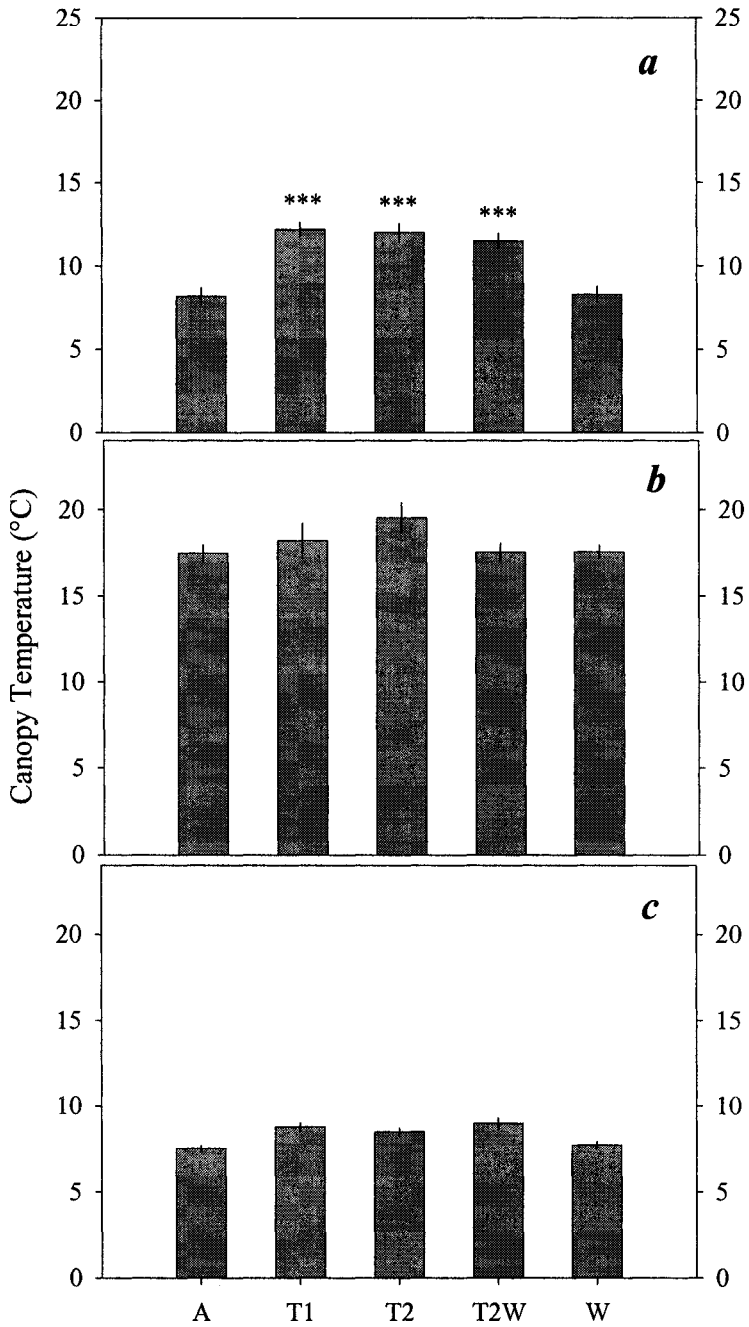

Figure 3.3. Canopy temperatures (°C) under diffuse ambient radiation (*a*), direct ambient radiation (*b*) and with wind speeds of 5 m/s (*c*). Statistical comparisons are against ambient (*P< 0.05, **P< 0.02, ***P< 0.001). Bars are 1.0 SE.

significant positive correlation (Figure 3.4). During the snow-free season, near surface soils (2 cm) were significantly warmer than ambient conditions in T1 (+1.2°C, P= 0.0073), T2 (+2.6°C, P<.0001) and T2W plots (+1.9°C, P<.0001). The effect of IR treatments on soil surface temperatures varied systematically over the diurnal cycle, with the greatest near surface (2 cm) warming early in the morning and the least in late afternoon (Figure 3.5). Soil temperatures at 2 cm in ambient and T2 plots were strongly correlated with wind speeds on hourly time-steps (Figure 3.6), but the slope of the correlation was significantly reduced in the T2 plots (P<.0001). Consequently, the magnitude of soil warming was significantly correlated with wind speed on hourly time-steps (r^2= 0.93, P<.0001). Soil temperatures at 2 cm in ambient and T2 plots were also strongly correlated with wind speeds on daily time-steps (Figure 3.7). High winds reduced shallow soil temperatures in the warmed plots to a greater extent than in ambient plots. Consequently, the magnitude of soil warming was significantly correlated with wind speed on daily time-steps (r^2= 0.60, P<.0001). Because the greatest soil warming was observed under low winds and relatively warm ambient soils, there was a significant positive correlation between ambient soil temperatures and the magnitude of soil warming (r^2= 0.31, P<.0001) (Figure 3.8)

Diurnal variation in soil warming and the overall magnitude of the effect declined with depth. The rate of decline in soil warming with depth differed across treatments. At 2 cm, T2 plots were consistently warmed to a greater extent than T2W plots (Figure 3.9). This pattern disappeared at 5 cm and at 10 cm, T2W plots were consistently warmer than T2 plots. There was no evidence that weekly irrigation affected soil temperatures in W plots at any depth.

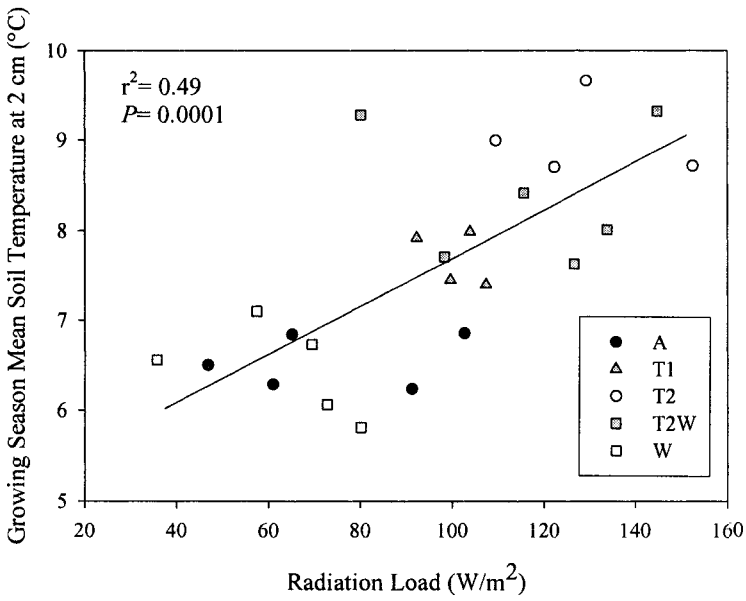

Figure 3.4. Simple linear regression of growing season mean soil temperatures at 2 cm (°C) on July 1, 2004 radiation at the soil surface.

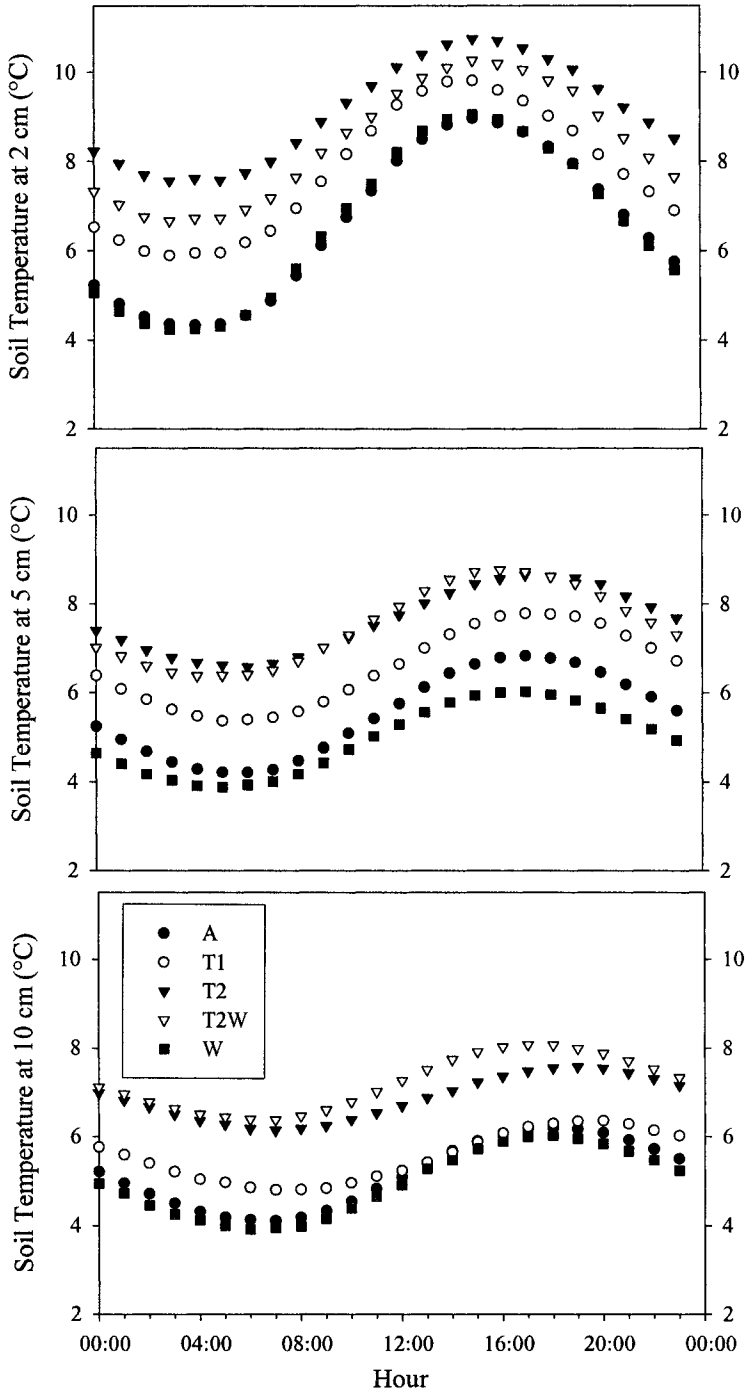

Figure 3.5. Diurnal soil temperature curves (°C) at 2, 5 and 10 cm in experimental plots between June 8 and August 19, 2004.

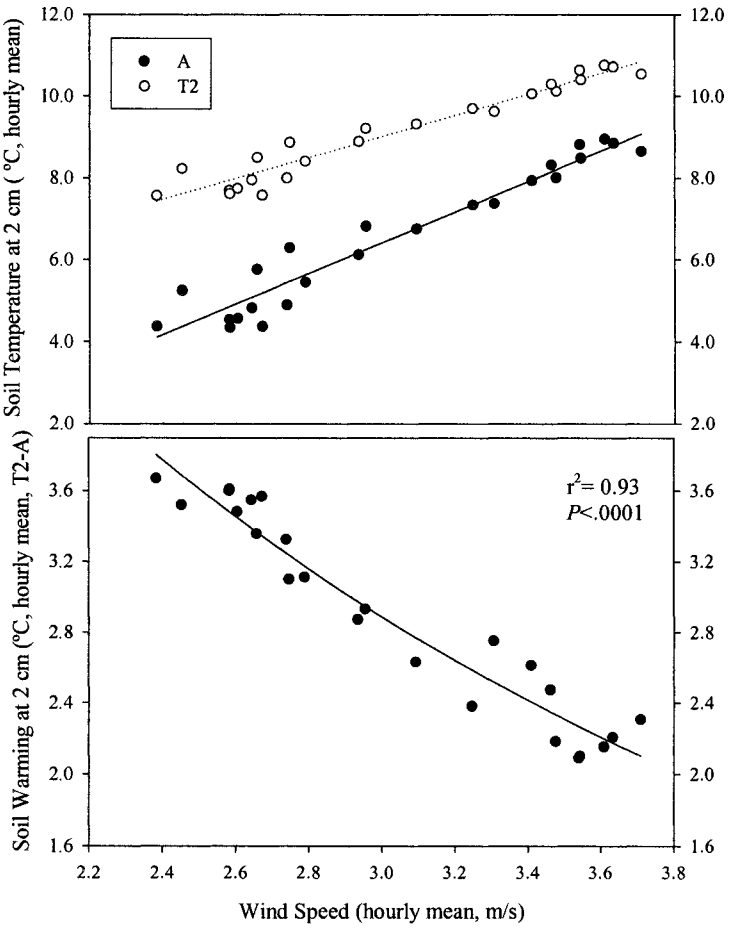

Figure 3.6. Simple linear and quadratic regressions of soil temperatures at 2 cm (˚C, A and T2) and soil warming at 2 cm (˚C, T2-A), respectively, on wind speeds (m/s) at hourly time-steps.

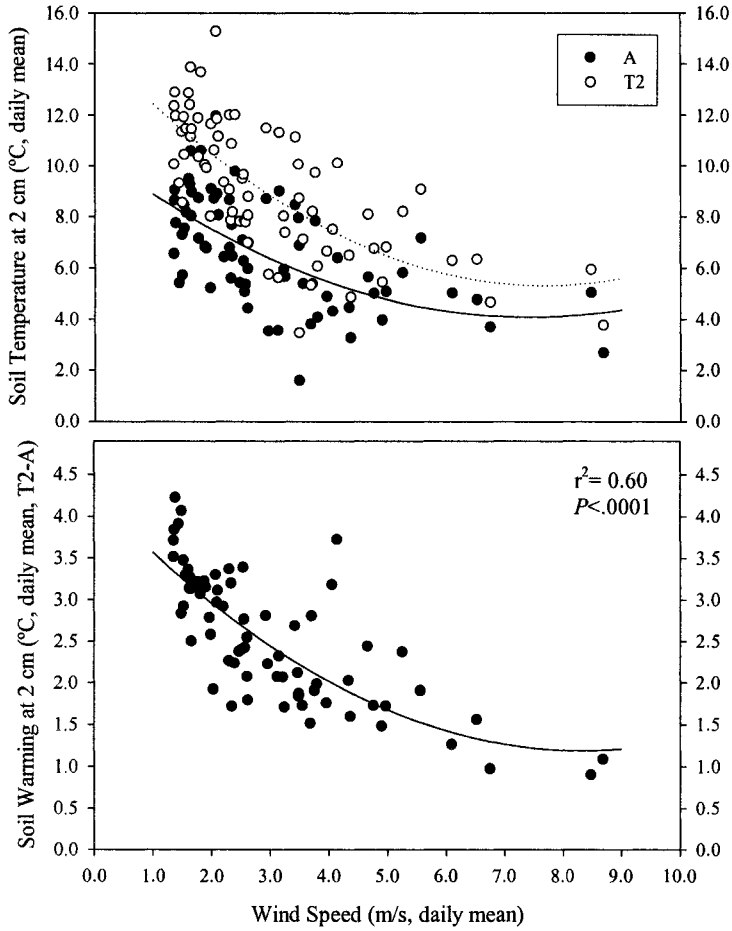

Figure 3.7. Quadratic regressions of soil temperatures at 2 cm (°C, A and T2) and soil warming at 2 cm (°C, T2-A) on wind speeds (m/s) at daily time-steps.

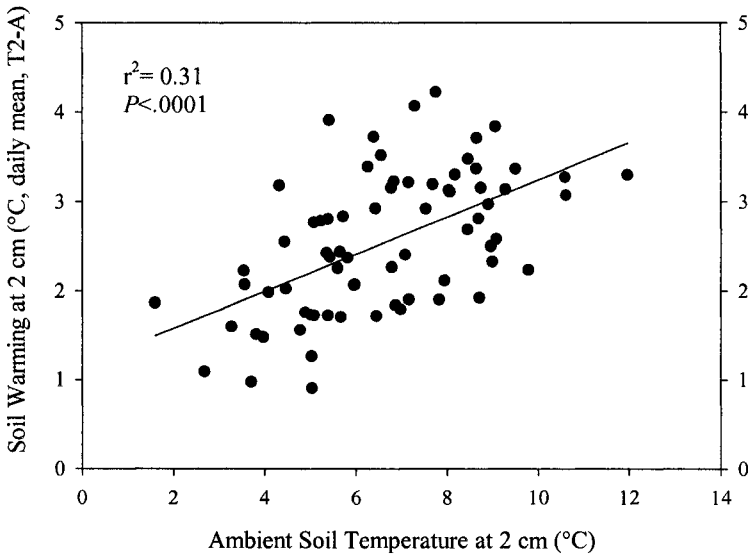

Figure 3.8. Simple linear regression of soil warming at 2 cm (°C, T2-A) on ambient soil temperatures at 2 cm (°C).

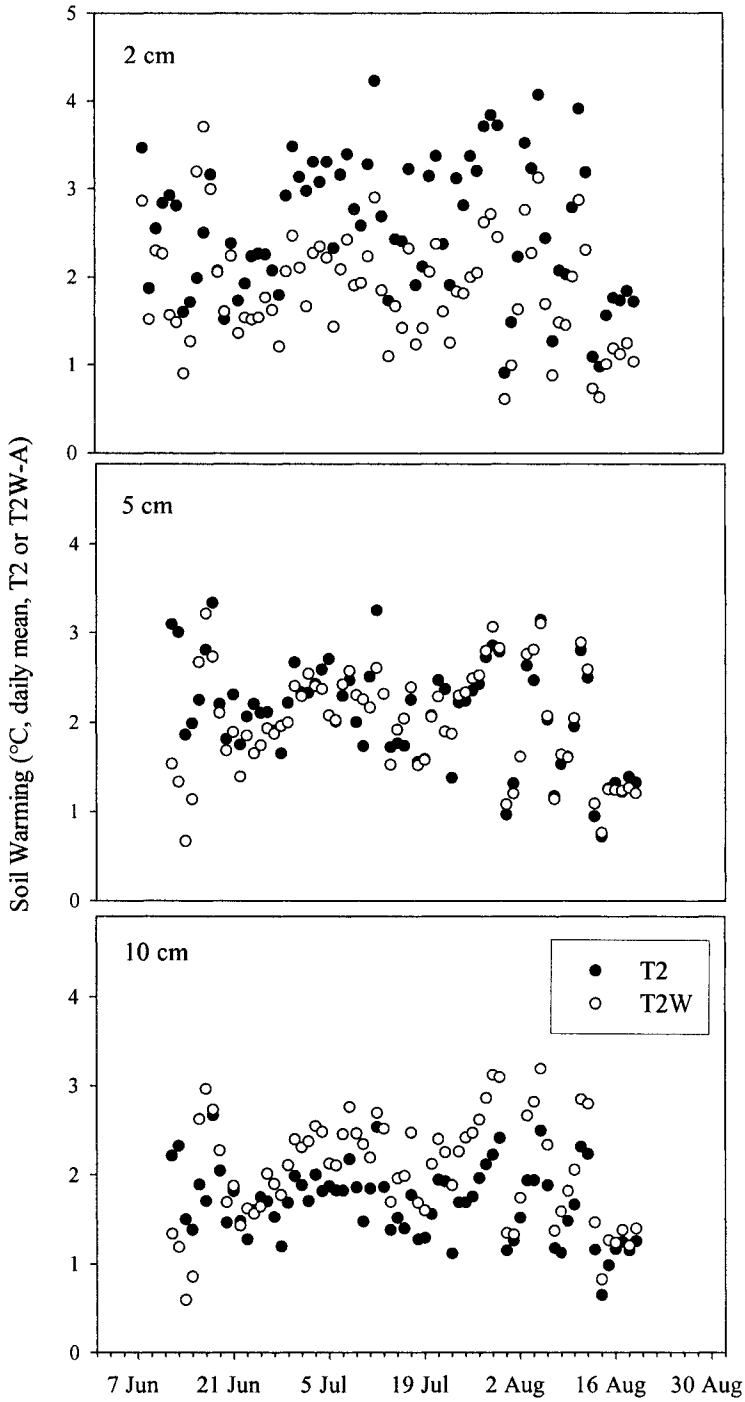

Figure 3.9. Soil warming (daily mean treatment-ambient) at 2, 5 and 10 cm in T2 and T2W plots between June 8 and August 19, 2004.

There was no evidence that radiation additions or irrigation affected volumetric soil water content (0-12 cm, measured weekly) at any time during the snow-free season (Figure 3.10). Weekly measurements of ambient soil water content revealed significant differences between successive sampling dates on just two occasions. There were significant declines between June 10 and 17 (P= 0.0004) and between June 23 and 30 (P= 0.0044), but no evidence of differences amid successive dates after June 30.

There was no evidence that soil water contents were greater than ambient one hour after irrigation with 5.0 mm on August 8, 2004, whether the measurements were made on a volumetric (0-12 cm, P= 0.8555) or gravimetric basis (0-5 cm, P= 0.9999) (Figure 3.11). The site received 7.4 mm of rain between the one-hour and 24-hour samples and 2.8 mm of rain between the 48-hour and 72-hour samples. There was no evidence of an increase in ambient volumetric or gravimetric soil water content following either the 7.4 mm (P= 0.1666, 0.9115) or 2.8 mm events (P= 0.8788, 0.9999).

Discussion

Radiation additions in T1, T2 and T2W plots were approximately 30, 60 and 50 W/m^2 respectively. These additions are greater than those reported for a Colorado montane meadow (~22 W/m^2, Harte et al. 1995), similar to those applied in high arctic wet tundra (~50 W/m^2, Nijs et al. 2000), less than those used in a California annual grassland (~80 W/m^2, Shaw et al. 2002), and probably less than those used to manipulate Minnesota peatland mesocosms (78 and 191 W/m^2 indoors, Bridgham et al. 1999). All of these additions are greater than expected with increased greenhouse gas forcing because they compensate for loss of thermal energy to the surrounding environment.

There was no evidence of warming in open air, 20 cm above the plant canopy, where

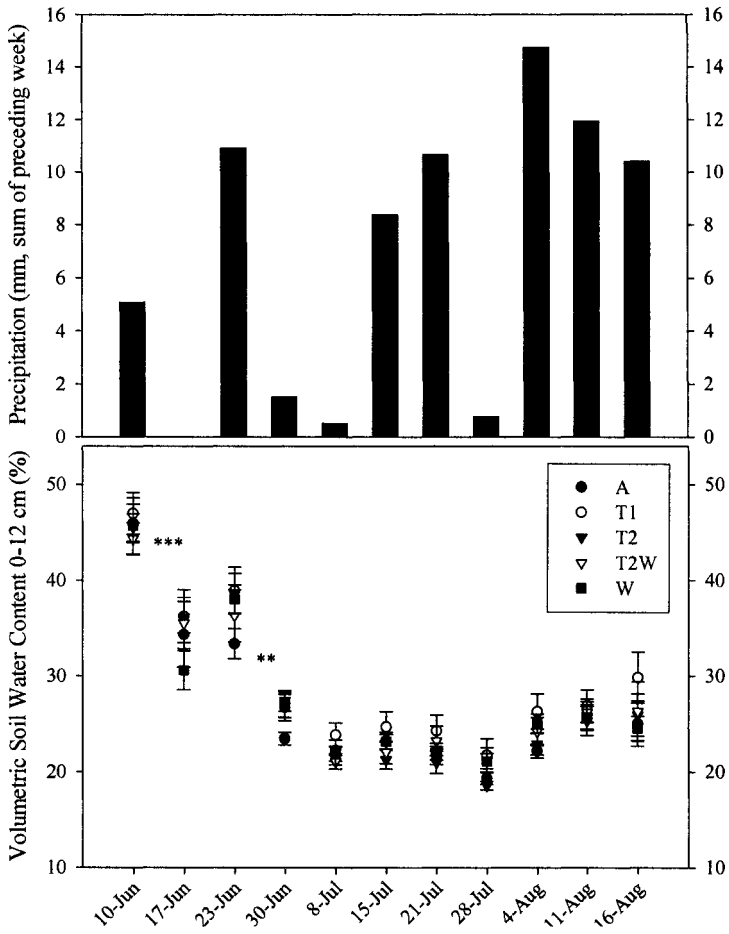

Figure 3.10. Seasonal pattern of volumetric soil water content (%) in the experimental plots and precipitation during the preceding week (mm). Statistical comparisons are of successive sampling dates in the ambient plots (*P< 0.05, **P< 0.02, ***P< 0.001). Bars are 1.0 SE.

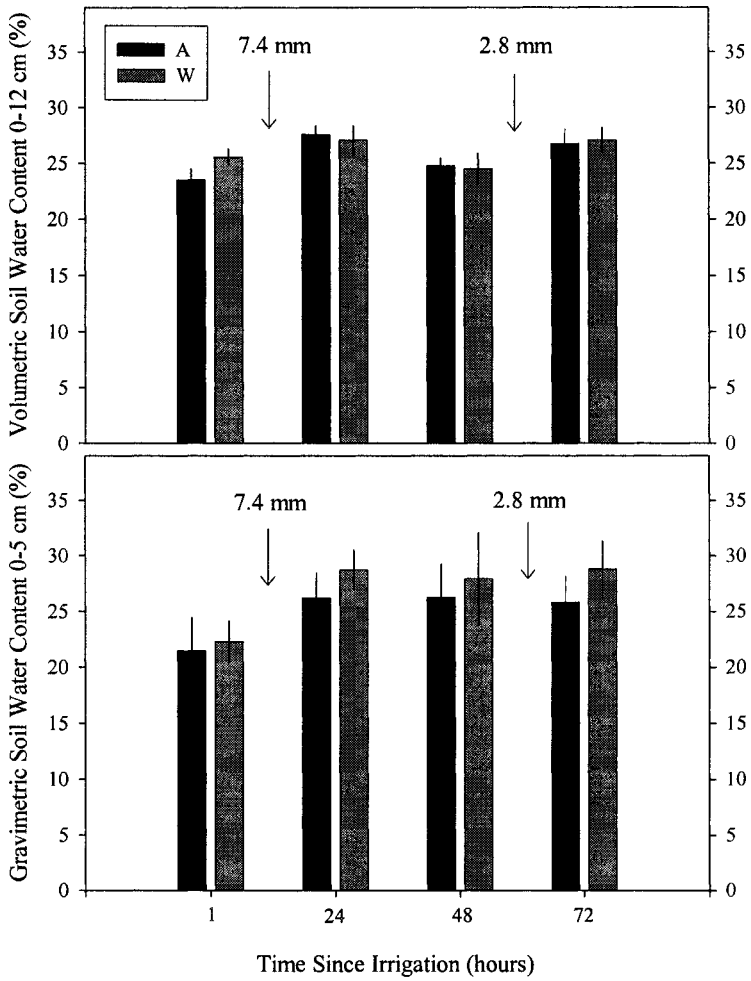

Figure 3.11. Gravimetric (0-5 cm) and volumetric (0-12 cm) soil water content (%) in ambient (A, n= 4) and irrigated (W, n= 4) plots 1 hour, 24, 48 and 72 hours after irrigation with 5.0 mm. Statistical comparisons are across treatments within sampling times (*P< 0.05, **P< 0.02, ***P< 0.001). Bars are 1.0 SE.

canopy structure is presumed to have a limited effect on air temperatures. This observation is consistent with results in a montane meadow (Saleska et al. 1999), but contrasts with reports from high arctic graminoid tundra (Nijs et al. 2000) and tallgrass prairie (Wan et al. 2002). Open air warming does not occur because there are too few molecules of radiatively-active gases between an IR lamp and the plant canopy. Different reports of IR lamp effects on air temperatures may reflect differences in canopy structure and measurement height. In both cases where air warming was reported, air temperature measurements were taken within the canopy of graminoid-dominated ecosystems, which tend to be decoupled from conditions in the overlying atmosphere. In the montane meadow, air temperatures were measured at various heights (J. Harte personal communication) in a rough canopy that may be well coupled with the atmosphere.

The lack of open air warming is indicative of an important pathway for loss of supplemental energy from experimental plots. Loss of supplemental energy from the experimental plots occurs by convective transfer to the boundary layer, followed by advective loss to the surrounding atmosphere. There were negative exponential correlations between wind speed and the magnitude of soil warming on both hourly and daily time-steps. Similarly, there was no evidence of a difference between canopy temperatures in ambient and warmed plots when measurements were taken under breezy conditions (~5 m/s). High winds depress the boundary layer, increase the rate of convective heat transfer from the ecosystem to the atmosphere and remove heat by advection. Canopy and soil warming in the absence of air warming increased the temperature gradient from the ecosystem to the atmosphere, thereby increasing

convective heat loss, particularly under high winds and a thin boundary layer. While the wind effect on ecosystem warming is a natural process, the magnitude of the effect was probably exacerbated by the absence of air warming.

High winds reduced shallow soil temperatures in warmed plots to a greater extent than in ambient plots. Because the greatest soil warming was observed under low winds and relatively warm ambient soils, there was a significant positive correlation between ambient soil temperatures and the magnitude of soil warming. Harte et al. (1995) found a similar positive correlation between ambient soil temperatures and the magnitude of soil warming in the drier upper zones of their plots. They suggested the positive correlation was a consequence of synergism between IR lamp-induced soil drying and increasing solar radiation near mid-day, when ambient soil temperatures are warmer. In my study, there was a very weak correlation between daily soil warming and daily maximum solar radiation (r^2= 0.07, P= 0.0200). Furthermore, there was no evidence of an IR lamp effect on soil water content. These observations suggest that synergistic effects of lamp-induced soil drying and rising solar radiation were not active at my site.

In many parts of the globe, daily minimum temperatures have increased at nearly twice the rate of daily maximum temperatures (IPCC 2001). Infrared warming reduced the diurnal temperature range (DTR) of shallow soils, with the greatest warming observed in the early morning hours. This observation contrasts with other infrared warming experiments, where increases in the DTR of near surface soil have been reported (Harte et al. 1995, Wan et al. 2002). While the change in the DTR of shallow soils at my site is consistent with expectations in a warmer climate, global reductions in

the DTR have been attributed to increases in atmospheric moisture (IPCC 2001). Atmospheric moisture was not manipulated in my study.

Irrigation affected the vertical distribution of supplemental heat. There was greater warming in T2 than T2W plots at 2 cm, similar warming at 5 cm and greater warming in T2W than T2 plots at 10 cm. Greater warming in T2W plots at depth occurred despite lower mean radiation additions in T2W plots. In the High Arctic, cryoturbation can transport pockets of soil carbon to depth. For instance, approximately 40% of soil carbon in vegetated areas occurs below 25 cm at my site (Horwath and Sletten unpublished data). An increase in summer precipitation would conduct additional heat to depth and may stimulate mineralization of deep soil carbon. Deep soil carbon may be particularly prone to mineralization if increases in precipitation and temperature coincide in a changing climate.

There was no evidence that supplemental radiation reduced soil water contents, in contrast with the results of previous infrared warming experiments (Harte et al. 1995, Wan et al. 2002). The absence of such a response is apparent in the direct measurements of soil water content and in the observation that T2W plots did not fall below the regression line that relates radiation load to soil temperatures at 2 cm. Under ambient conditions, there were significant changes in soil water content among successive sampling dates on just two occasions, early in the growing season. The presence of an impermeable frozen layer near the soil surface probably led to retention of soil water that would have otherwise drained. It is probable that early season declines in soil water content correspond with periods of rapid downward progression of the frozen soil interface.

Irrigation had no effect on soil water content near the soil surface (0-12 cm), even when measurements were made just one hour following application, despite the importance of supplemental water in distributing heat to depth. The particle size distribution of the near surface soil (0-12 cm) at my site is 65-75% sand, 20-35% silt and 5-8% clay. The organic carbon content over the same depth is low (1.7-2.0%), as is the volumetric water holding capacity (11-13%), when estimated using texture (Saxton et al. 1986). Soil texture at my site is within the range observed in prostrate dwarf-shrub, herb tundra elsewhere near Pituffik, where soils range from 63-80% sand, 17-33% silt and 2-4% clay.

The apparent resistance of soil water content to change has important implications for the system's surface energy balance in a changing climate. Unless climate change leads to increases in soil organic matter, and thus water holding capacity, changes in precipitation may not affect the proportion of energy transferred from the surface to the atmosphere as sensible versus latent heat (i.e., the Bowen Ratio).

Conclusions

Radiation and water manipulations affected plant canopy and soil microclimates in ways that were both similar to and decidedly different than climate change experiments in other ecosystems. As anticipated, long-wave radiation additions increased canopy and soil temperatures. Soil warming penetrated to depths greater than 10 cm, as reported in previous IR lamp experiments, and did so to the greatest extent when radiation additions were combined with supplemental water. It was a surprise to find that wind speeds exerted such a strong control over soil temperatures and the magnitude of soil warming on hourly and daily time-steps. Wind is a variable that has received

comparatively little attention in the context of climate change – vegetation interactions. The results of my study suggest that wind warrants more attention.

The apparent resistance of soil water content to change also came as a surprise. Soil drying may be the most consistent effect of previous IR lamp experiments, second to soil warming. There was evidence of a change in soil water contents with either supplemental radiation or supplemental water (~2x ambient precipitation). The apparent resistance of near surface soil water content to change may buffer the ratio of sensible to latent heat flux in a changing climate.

The frequency with which I rejected simple, intuitive hypotheses about the effects of greater radiative forcing and precipitation on canopy and soil microclimates highlights the importance of detailed microclimate monitoring in field climate change experiments. Microclimate responses observed in one ecosystem may not hold in other ecosystems.

CHAPTER IV

Variation in leaf physiology across experimental and natural gradients in the High Arctic: test of a dual isotope ($\delta^{13}C$ and $\delta^{18}O$) conceptual model

Introduction

Stable isotope ratios of carbon and oxygen ($\delta^{13}C$ and $\delta^{18}O$) can provide insights into both contemporary (Yakir and Sternberg 2000, Dawson et al. 2002, Ehleringer at al. 2002) and retrospective (McCarroll and Loader 2004) plant and ecosystem physiology, making them an ideal tool for studies of vegetation responses to global change. The use of stable isotopes to study the effects of climate change on vegetation may be particularly valuable in the Arctic, where recent and projected changes in temperature and precipitation are amplified relative to temperate and tropical regions (Serreze et al. 2000, ACIA 2004). In this context, investigators have used stable isotopes of carbon, oxygen and hydrogen to reveal the effects of long-term climate variability (Beerling and Rundgren 2000), inter-annual variability in the seasonality of precipitation (Welker et al. 1995, in press), experimental warming (Welker et al. 1993, 2004, Michelsen et al. 1996), shading (Michelsen et al. 1996), nutrient application (Michelsen et al. 1996) and changes in snow depth (Sullivan and Welker in review) on the gas exchange physiology of arctic plants. The mechanisms that underlie carbon isotope discrimination during photosynthesis are relatively well understood (Farquhar et al. 1982, 1989a, 1989b). Oxygen isotope ratios in plant material have been measured less frequently, and while

much progress has been made recently (see Barbour et al. 2005 for review), some facets of oxygen isotope theory require further testing under field conditions, particularly regarding the relationships between $\delta^{18}O$ and $\delta^{13}C$.

Carbon Isotope Theory

The carbon isotope ratio of plant material is depleted relative to that of atmospheric CO_2 as a result of two fractionation steps during carbon assimilation. The first fractionation step occurs during CO_2 diffusion into the leaf, as $^{13}CO_2$ diffuses more slowly than $^{12}CO_2$. The second fractionation step occurs during carboxylation, as ribulose 1,5-bisphosphate carboxylase/oxygenase reacts more readily with $^{12}CO_2$. Carbon isotope discrimination during photosynthesis is, therefore, expressed as follows (Farquhar et al. 1982, 1989a):

$$\delta^{13}C_p = \delta^{13}C_a - a - (b - a)(c_i/c_a), \qquad \text{(eq. 4.1)}$$

where $\delta^{13}C_p$ and $\delta^{13}C_a$ are the carbon isotope ratios (expressed relative to the Pee Dee Belemnite standard) of leaf tissue and atmospheric CO_2, respectively, a is fractionation during diffusion (4.4 ‰), b is fractionation during carboxylation (27 ‰) and c_i and c_a are CO_2 concentrations in leaf inter-cellular spaces and in the atmosphere, respectively. Equation 1 indicates that, when $\delta^{13}C_a$ is known, c_i/c_a drives variation in $\delta^{13}C_p$. Inter-cellular CO_2 concentrations may vary with either photosynthetic capacity (A_{max}) or stomatal conductance (g_s). Therefore, c_i/c_a is indicative of the balance between carbon gain and water loss.

Oxygen Isotope Theory

The $\delta^{18}O$ value of meteoric water varies with temperature, altitude, latitude, continentality, and precipitation intensity (Dansgaard 1964, Rozanski et al. 1982,

Rozanski, et al. 1992, 1993, Welker 2000, Kohn and Welker 2005). In the Arctic, highly seasonal air temperatures give rise to a $\delta^{18}O$ pattern in precipitation that is distinctly seasonal, with winter precipitation that is depleted relative to summer rain (Rozanski et al. 1993, Cooper et al. 1993, 1996, Sullivan and Welker in review).

The $\delta^{18}O$ value of soil water reflects a weighted average of $\delta^{18}O$ in precipitation and near surface evaporative enrichment. Oxygen isotopes in soil water are not fractionated during uptake by roots. Thus, xylem water $\delta^{18}O$ is equivalent to source water $\delta^{18}O$ (Ehleringer and Dawson 1992). Leaf water is enriched above xylem water during transpiration. Leaf water enrichment (LWE) is positively related with the difference between source water $\delta^{18}O$ and atmospheric water vapor $\delta^{18}O$, positively related with the vapor pressure difference between the atmosphere and leaf inter-cellular spaces (VPD) and negatively related with transpiration rate (E) (Flanagan et al. 1991, Farquhar and Lloyd 1993). When leaf water reaches the chloroplasts, carbonic anhydrase (CA) catalyzes the near complete exchange of $^{18}O/^{16}O$ with CO_2. Further enrichment occurs during biosynthesis. Cellulose, for instance, is approximately 27 ‰ enriched relative to water at the site of synthesis, as a result of exchange between carbonyl-oxygen and water (Sternberg and DeNiro 1983, Sternberg 1989, Yakir and DeNiro 1990). Leaf cellulose, therefore, carries a $\delta^{18}O$ value that reflects both source water $\delta^{18}O$ and LWE.

Combining Carbon and Oxygen Isotope Ratios

LWE varies with E and the VPD. The former is closely correlated with g_s and the later is an important determinant of g_s. Two processes limit LWE when rates of E and g_s are relatively high. Transpiration reduces leaf temperature and the water vapor pressure of leaf intercellular spaces, thereby reducing the vapor pressure difference between

intercellular spaces and the atmosphere. Transpiration also affects mixing of water within the leaf. When rates of E are relatively high, mixing is dominated by the convective flux of unfractionated water to the sites of evaporation; this tends to reduce leaf water enrichment. When rates of E are relatively low, mixing is dominated by the backward diffusion of $H_2{}^{18}O$ into the leaf; this tends to increase leaf water enrichment. The ratio of the convective flux to and the backward diffusion of enriched water is described by a Péclet effect (Farquhar and Lloyd 1993). Building upon the recognition that leaf water enrichment varies with E and g_s, several authors have suggested changes in the magnitude of leaf water ^{18}O enrichment may enable differentiation between changes in leaf $\delta^{13}C$ (c_i/c_a) that are driven by changes in g_s and those that are driven by changes in A_{max} (Farquhar et al. 1989b, Farquhar and Lloyd 1993, Yakir and Israeli 1995, Saurer et al. 1997, Farquhar et al. 1998, Barbour and Farquhar 2000, Scheidegger et al. 2000, Barbour et al. 2005).

Relatively few investigations have examined both carbon and oxygen isotopes of plant material in the field (Sternberg et al. 1989, Yakir and Israeli 1995, Saurer et al. 1997, Scheidegger et al. 2000, Barbour et al. 2000, Barbour et al. 2002, Cernusak et al. 2005, Sullivan and Welker in review). Successful application of the dual isotope approach requires isolation of the leaf water signal, which varies with a driver and a strong correlate of g_s, and validation of isotope-based inferences with gas exchange measurements. Isolating leaf water ^{18}O enrichment requires direct measurements of leaf water $\delta^{18}O$, constant source water $\delta^{18}O$, or measurements of both stem water $\delta^{18}O$ and leaf material $\delta^{18}O$. The oxygen isotope composition of leaf material is typically

expressed as enrichment above source water ($\Delta^{18}O$), when both stem water $\delta^{18}O$ and leaf material $\delta^{18}O$ are known (Barbour et al. 2004).

In this study, I examined carbon and oxygen isotope ratios of leaf α-cellulose in *Salix arctica* within plots subjected to experimental energy inputs and along natural gradients of soil temperature and soil water content. I made corresponding measurements of stem water $\delta^{18}O$ and leaf-level gas exchange to isolate the leaf water signal and test my isotope-based inferences respectively. The High Arctic growing season is limited to less than 90 days and *S. arctica* maintains fully expanded leaves for as few as 40 days. Therefore, good temporal coverage could be obtained with relatively few measurements of leaf-level gas exchange and stem water $\delta^{18}O$, making it an ideal location for a test of the dual isotope approach.

Materials and Methods

Study Species

Salix arctica is a widespread, woody, long-lived arctic-alpine dwarf willow. It is deciduous, dioecious and occurs in a wide range of habitats throughout much of the circumpolar Arctic. *S. arctica* exhibits an unusual pattern of shoot development, where ramets develop as solely vegetative or reproductive, producing catkins as well as leaves. Leaves are produced as a single cohort that expands within two weeks of snowmelt. *S. arctica* has been the subject of detailed physiological investigations (Dawson and Bliss 1989a, 1989b, 1993) and climate change experiments (Jones et al. 1999) in the Canadian High Arctic.

Site and Treatment Descriptions

Measurements were taken at three sites near Pituffik (Thule), Greenland: a prostrate dwarf-shrub, herb tundra (PDSHT) (CAVM 2003) and north- and south-facing hill slopes. Mean annual air temperature and precipitation in Pituffik were -11.6°C and 11.6 cm respectively, between 1978 and 2000. The North Mountain PDSHT (76° 33'N, 68° 34'W) is the site of multivariate climate manipulations that have been described extensively elsewhere (Sullivan et al. in review). In this study, leaf physiology was investigated under ambient conditions and beneath two levels of supplemental radiation supplied using overhead infrared lamps (T1: ~30 W/m^2 and T2: ~50 W/m^2). Vascular plant cover at North Mountain is approximately 50% and the soils are Typic Haploturbels (Soil Survey Staff 1998). Areas without plant cover are characterized by frost boils, which typically grade from bare soil near the active center to 100% cryptogamic cover at the periphery.

Belt transects (15 x 100 m) were established on a north-facing hill slope (76° 31' N, 68° 26' W) and a south-facing hill slope (76° 34' N, 68° 39' W). Both transects were established on basaltic outcrops, although the south-facing outcrop is overlain by a deeper carbonate layer. The belt transects were designed to encompass three different hydrologic regimes that correspond with three distinct ecosystems and were included in the study to maximize observed differences in leaf physiology. The belt transects were not designed to describe differences in leaf physiology on north- versus south-facing slopes in general. The xeric upper zones are PDSHT, where vascular plant-cover ranges from 25-50%. The mesic middle zones are hemiprostrate dwarf-shrub tundra (HDST), dominated by *Cassiope tetragona*, with vascular plant-cover that approaches 100%. The

hydric lower zones are sedge/grass moss wetlands (SGMW), where soil surface is a mosaic of moss mats and irregular sedge hummocks. *S. arctica* is generally confined to the tops and sides of the hummocks.

In the interest of parsimony, the treatments at the North Mountain PDSHT are referred to as A, T1 and T2, while the hydrologic zones/ecosystems at the north- and south-facing hill slopes are preceded by their aspect throughout the text (e.g., S-PDSHT refers to the xeric upper zone of the south-facing transect) (Table 4.1).

Microclimate Monitoring

Hourly air temperatures and relative humidity at 20 cm were monitored in the HDST zones of the belt transects and under ambient conditions at North Mountain with HOBO Pro Series loggers housed in radiation shields (Onset Computer Corp., Bourne, MA). Hourly soil temperatures at 5 and 10 cm were monitored in two replicates of each zone or treatment at each site with Hobo outdoor 4-channel external temperature loggers (Onset Computer Corp., Bourne, MA). Measurements began on June 13, immediately following snowmelt, and ceased on August 20, before snow pack development. The SGMW sites have a deeper winter snow pack and a shorter growing season. Therefore, soil temperature measurements began later at these sites: on June 26 at S-SGMW and on June 30 at N-SGMW.

Soil water content was measured in the upper 12 cm (n= 4) during gas exchange measurements in each zone or treatment at each site with a hand-held HydroSense TDR probe (Campbell Scientific, Logan, UT). Snow depth measurements were made in each zone of the belt transects and under ambient conditions at North Mountain in the first week of April 2004.

Soil cores (3.8 cm diameter) were taken to 12 cm depth in each zone of the belt transects and under ambient conditions at North Mountain in late August 2004. Samples were homogenized in the laboratory and 5.0 g sub-samples of soil (<2 mm) were immersed in 25.0 mL of DI water, stirred and allowed to stand for 30 minutes. Solution pH was measured using a WTW Multiline 340i meter equipped with a SenTix 41-3 pH probe (Geotech Environmental Equipment, Inc., Denver, CO).

Precipitation Measurement and Sampling

Precipitation during the 2004-growing season was measured using plastic rain gauges installed at ground level. Gauges were read immediately following cessation of an individual event. Nalgene bottles (125 mL) fit with nylon filters and 10 cm diameter funnels were used to collect samples from each event. Samples were collected immediately following cessation of precipitation and frozen until analysis to reduce the potential for evaporative enrichment. Snow cores (3.8 cm diameter) were collected from each zone of the belt-transects and under ambient conditions at North Mountain in early April 2004. Snow samples were sealed in Ziploc bags, melted in the laboratory, transferred Nalgene bottles and frozen until analysis.

Stable Isotope and Leaf Nutrient Analyses

On three dates during the 2004-growing season (early, mid- and late July), a 15 m tape was laid perpendicular to the long axis of the belt transects and four female, vegetative *S. arctica* ramets were randomly selected. At North Mountain, ramets were selected arbitrarily from four replicates of each treatment (A, T1 and T2). Leaf material was removed from the ~5 cm stem segments and placed in coin envelopes. Stem samples were immediately placed in glass dram vials fit with Teflon cap liners and parafilm was

wrapped around the cap/vial junctions. Samples were placed on ice in a portable field cooler and frozen upon return to the laboratory. Leaf samples were dried for 24 hours at 60°C then ground to a fine powder.

Stem water was extracted by cryogenic vacuum distillation (Ehleringer and Osmond 1989, Ehleringer et al. 2001). Frozen stem samples were diced to increase surface area and submersed in liquid nitrogen (-196°C) during evacuation of the vacuum line. Following evacuation, individual samples were isolated and incrementally heated to vaporize the stem water. Water vapor was trapped in a tube cooled by liquid nitrogen. Samples were eliminated from subsequent analyses if <98% of stem water was extracted or if there was evidence of a vacuum leak during extraction.

To measure stable oxygen isotope ratios in stem waters, snow and summer precipitation, 0.2 mL of each sample was transferred to a 1.0 mL glass vial, aspirated with 10% CO_2 and 90% He in a glove bag and equilibrated for 10 hours at 30°C. The isotopic composition of CO_2 in the head-space was measured using a multi-prep sampler interfaced with a dual inlet VG-Optima stable isotope ratio mass spectrometer (Micromass UK Ltd., Manchester, Lancashire, UK) (Epstein and Mayeda 1953). De-ionized water (DI), cooked water (CW) and water from Cameron Pass, Colorado (CP), of known isotopic composition, were included every five samples. The standard deviations of these embedded standards were as follows: DI= 0.16‰, CW= 0.25‰ and CP= 0.21‰. Oxygen isotope ratios are reported relative to the Vienna Standard Mean Ocean Water (VSMOW) standard as follows:

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000, \qquad \text{(eq. 4.2)}$$

where δ is reported in per mille (‰) and R_{sample} and $R_{standard}$ are the isotope ratios of the sample and standard respectively.

Stable carbon and oxygen isotope ratios were analyzed in leaf α-cellulose. Leaf holocellulose was isolated using the method described by Leavitt and Danzer (1993), where toluene and ethanol are used to remove lipids in a soxhlet apparatus, boiling water is used to remove soluble sugars and sodium chlorite and glacial acetic acid are used to remove lignin and proteins. Holocellulose was subsequently reduced to α-cellulose by washing the samples in 17% (w/v) NaOH (Roden and Ehleringer 1999).

Carbon and nitrogen concentrations in bulk leaf material and carbon isotope ratios in leaf α-cellulose were analyzed using a Carlo Erba elemental analyzer (Thermo Electron Corporation, Milan, Italy) interfaced with an Isochrom stable isotope ratio mass spectrometer (Micromass UK Ltd., Manchester, Lancashire, UK). Oxygen isotope ratios in leaf α-cellulose were measured using a EuroVector Pyrolysis unit (EuroVector, Milan, Italy) interfaced with a VG-Optima stable isotope ratio mass spectrometer (Micromass UK Ltd., Manchester, Lancashire, UK) operated in continuous flow mode. One vacuum oil standard was included every 10 samples and two cellulose standards were included every six samples in the carbon and oxygen analyses, respectively. Carbon and oxygen isotope ratios are reported in δ notation relative to the Pee Dee Belemnite (PDB) and VSMOW standards, respectively. The standard deviation of the embedded vacuum oil standards was 0.16‰, while that of the cellulose standards was 0.48‰.

Carbon isotope ratios can be expressed as discrimination relative to $\delta^{13}C$ of atmospheric CO_2 as follows (O'Leary 1993):

$$\Delta^{13}C = \frac{\delta^{13}C_a - \delta^{13}C_x}{1+\delta^{13}C_x/1000}, \qquad \text{(eq. 4.3)}$$

where $\delta^{13}C_a$ is that of atmospheric CO_2 and $\delta^{13}C_x$ is that of bulk leaf material or α-cellulose. Similarly, oxygen isotope ratios can be expressed as enrichment above source water (Barbour et al. 2004):

$$\Delta^{18}O = \delta^{18}O_x - \delta^{18}O_s, \qquad \text{(eq. 4.4)}$$

where $\delta^{18}O_x$ is that of α-cellulose and $\delta^{18}O_s$ is that the source (xylem) water.

Gas Exchange Measurements

Leaf-level gas exchange measurements were made using a LI-6400 open-flow portable photosynthesis system equipped with a CO_2 mixer (LI-COR Biosciences, Lincoln, NE). Sex (Dawson and Bliss 1989b, Jones et al. 1999) and reproductive investment (Dawson and Bliss 1993) are sources of variation in *S. arctica* leaf physiology. To reduce non-climatic variation, measurements were taken only on "sun leaves" of female vegetative ramets. To identify the irradiance level associated with maximum rates of net photosynthesis in *S. arctica*, light-response curves (n= 4) were measured at mid-day in each zone of the belt transects in late June, mid-July and early August 2003. On each sampling date, a 15 m tape was placed through the center of each zone, perpendicular to the long axis of the transect, and fully-expanded near apical leaves on female, vegetative shoots of *S. arctica* were selected. Measurements were taken using a constant flow rate of 400 μmol sec^{-1}, a leaf temperature of 15°C and a CO_2 concentration of 400 μmol/mol. Irradiance was progressively increased from 0 to 2400 μmol m^{-2} sec^{-1} at intervals of 400 μmol m^{-2} sec^{-1}. At least 5 minutes were allowed to pass before measurements were logged at each irradiance-level. The Mitscherlich model

(Potvin et al. 1990) provides a quantitative description of the photosynthetic light response curve using biologically relevant parameters:

$$A = A_{max}\left[1 - e^{-Aqe(PPFD-LCP)}\right], \qquad \text{(eq. 4.5)}$$

where A_{max} is the light-saturated net photosynthetic rate or photosynthetic capacity, A_{qe} is the apparent quantum yield, PPFD is photosynthetic photon flux density and LCP is the light compensation point. The Mitscherlich model was iteratively fit to each light response curve using the non-linear model (NLIN) procedure in SAS 9.1 (SAS Institute, Cary, NC), which generates estimates and confidence intervals for each parameter in the model. To identify the irradiance level that provided the best estimation of the model-fit A_{max}, correlations between net photosynthesis measurements at 1600, 2000 and 2400 μmol m^{-2} sec^{-1} and model-fit A_{max} were examined using the regression procedure in SAS 9.1 (SAS Institute, Cary, NC). Net photosynthesis measurements taken at 2000 μmol m^{-2} sec^{-1} provided the best estimate of model-fit A_{max} (r^2= 0.89, P<.0001).

Measurements of mid-day leaf-level gas exchange were taken on near apical leaves from female, vegetative shoots of *S. arctica* on a ramet adjacent to that harvested for isotopic analysis in each zone or treatment (n=4) at each of the three sites in early, mid- and late July 2004. Measurements were taken with the irradiance level set at 2000 μmol m^{-2} sec^{-1}, a constant flow rate of 400 μmol sec^{-1}, a leaf temperature of 15°C and a CO_2 concentration of 400 μmol/mol.

Leaves were harvested, dried for 24 hours at 60°C, scanned individually at 300 dpi and analyzed using unsupervised classifications in Erdas Imagine to determine projected leaf areas (Leica Geosystems, Atlanta, GA). On one occasion, ten leaves were harvested from each zone of the south-facing belt transect, scanned, dried and scanned

again to develop a correction for reductions in leaf area with drying. This exercise revealed a strong correlation between wet and dry leaf areas (r^2= 0.99, P<.0001).

Stem Water Potentials

Immediately following gas exchange measurements, mid-day water potentials were measured on an unbranched cut stem of a third adjacent ramet in each zone or treatment at each of the four sites with a Scholander-type portable pressure chamber (Soilmoisture Equipment Corp. Santa Barbara, CA). Care was taken to limit variation in stem diameters and leaf areas. Two observers were used for each measurement: one to monitor the cut stem with a magnifying glass and the other to read the pressure gauge.

Statistical Analyses

Correlations between leaf cellulose $\delta^{18}O$ and $\Delta^{18}O$, g_s and stem water $\delta^{18}O$, stem water $\delta^{18}O$ and soil temperature and g_s and soil temperature were examined using the regression (REG) procedure in SAS 9.1 (SAS Institute, Cary, NC). The slope of correlation between leaf cellulose $\delta^{18}O$ and $\Delta^{18}O$ was tested for deviation from unity by analyzing residuals (observed – predicted if slope= 1) in the REG procedure of SAS 9.1. Variation in A_{max}, g_s, c_i/c_a, stem water potentials and leaf nitrogen content across zones or treatments at the three study sites was examined using analysis of variance (ANOVA) in the general linear model (GLM) procedure of SAS 9.1. Comparisons of interest were made using Tukey's Honest Significant Difference (HSD).

Results

Microclimate

There were subtle variations in air temperatures (0.33°C) and vapor pressure deficits (0.012 kPa) across the sites between June 13 and August 20, 2004 (Table 4.1).

Table 4.1. Microclimate characteristics in each zone or treatment between June 13 and August 20, 2004 at the three study sites. Snow depth measurements were made on one date in early April 2004. Soil volumetric water contents (0-12 cm) were averaged over four replicates in each zone or treatment on three dates during the 2004-growing season (n= 12). SGMW sites were released from snow cover later than the other sites. S-SGMW temperature and RH data are for June 26 – August 20, while those for N-SGMW are for June 30 – August 20. Standard deviations appear within parentheses.

Site	Air Temp. (°C)	VPD (kPa)	Soil Temp. (5 cm, ° C)	Soil Temp. (10 cm, ° C)	VWC (%)	Snow Depth (cm)	Soil pH
N-SGMW	4.75	0.119	5.06 (0.01)	4.45 (0.01)	45.8 (5.8)	55.6 (4.5)	6.1 (0.1)
N-HDST	4.39	0.122	4.21 (0.32)	3.70 (0.01)	19.3 (1.1)	15.6 (4.9)	6.0 (0.1)
N-PDSHT	4.39	0.122	6.16 (0.07)	5.32 (0.20)	14.0 (1.2)	9.8 (5.4)	6.1 (0.2)
S-SGMW	4.57	0.129	5.85 (0.27)	5.09 (0.22)	31.8 (6.3)	29.9 (5.1)	6.2 (0.2)
S-HDST	4.18	0.131	4.12 (0.15)	3.76 (0.06)	47.7 (12.0)	19.3 (5.1)	6.1 (0.3)
S-PDSHT	4.18	0.131	5.47 (0.68)	4.86 (0.92)	25.3 (3.5)	7.9 (2.5)	6.8 (0.1)
NM-A	4.51	0.134	5.52 (0.22)	5.18 (0.33)	19.6 (4.5)	6.4 (3.4)	6.7 (0.1)
NM-T1	4.51	0.134	6.75 (0.24)	5.74 (0.20)	23.7 (2.4)	6.4 (3.4)	6.7 (0.1)
NM-T2	4.51	0.134	7.61 (0.84)	6.87 (0.75)	18.8 (2.6)	6.4 (3.4)	6.7 (0.1)

There was considerable variation in soil temperature at 5 cm (3.49°C) and 10 cm (3.17°C) across treatments and zones within the sites. The infrared lamps warmed soils at 5 cm by 1.23°C and 2.09°C and soils at 10 cm by 0.56°C and 1.69°C in the T1 and T2 treatments, respectively. At both hill slope sites, the coldest soils were observed in the HDST zones. The infrared lamps did not affect volumetric soil water contents. At the north-facing hill slope, volumetric soil water content was lowest in the PDSHT, intermediate in the HDST and highest in the SGMW zone. This pattern roughly mirrored variations in winter snow depth. The south-facing hill slope deviated from this pattern in the sense that the highest soil water contents were found in the HDST zone, where winter snow depths were intermediate between the PDSHT and SGMW zones.

Oxygen Isotope Ratios in Precipitation and Stem Waters

Snow collected in early April 2004 had an average $\delta^{18}O$ value of –28.14‰. Precipitation during the 2004-growing season was concentrated in July and August, with few measurable events during June (Figure 4.1). The amount-weighted $\delta^{18}O$ value of 2004 summer rain was –19.32‰. Source water was enriched relative to both winter snow and summer rain, with an average $\delta^{18}O$ value of –14.79‰ and a range of 2.67‰, across zones and treatments at the three sites (Figure 4.2). There was a non-significant trend toward source water enrichment with increasing radiation load at North Mountain, such that the most enriched source waters were found in the T2 plots (-13.61‰). There was no evidence of a trend in source water $\delta^{18}O$ at the north-facing transect, but there was trend of source water enrichment along the south-facing transect, where source water in S-PDSHT was depleted relative to source water in S-SGMW.

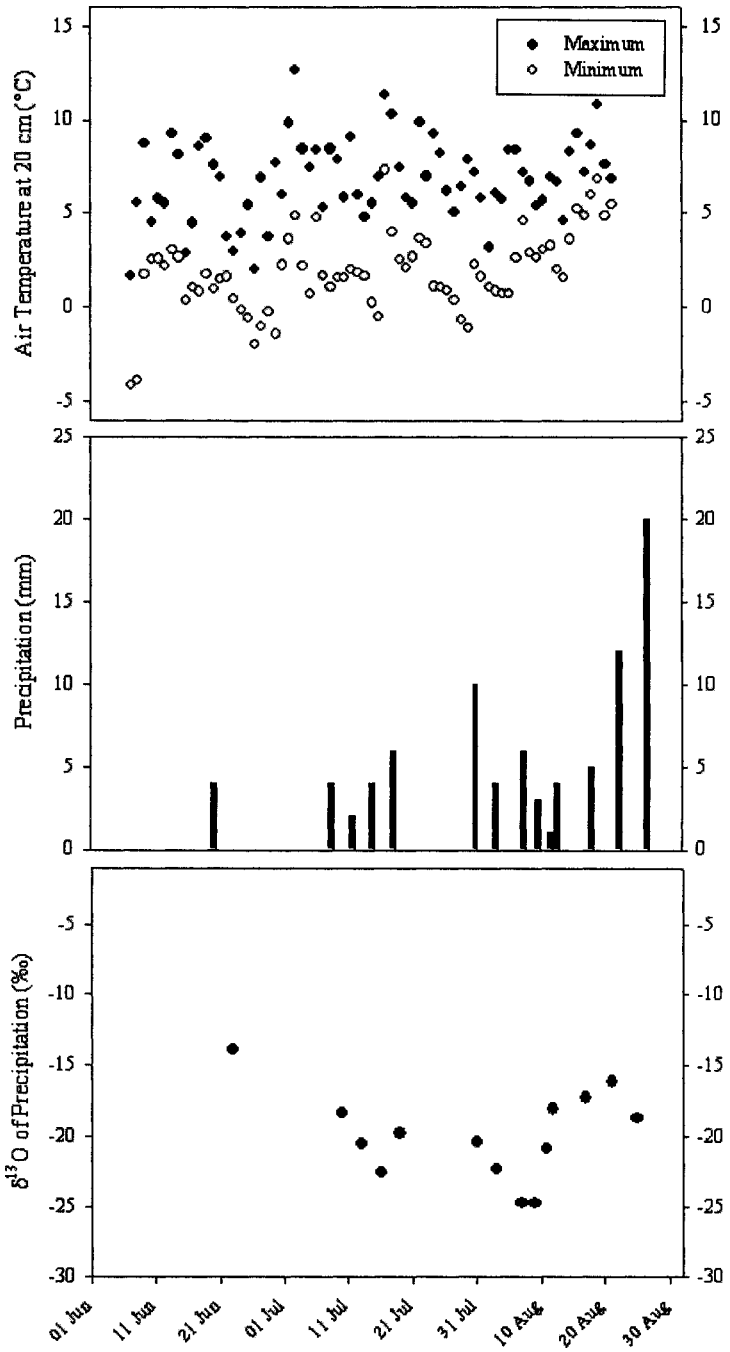

Figure 4.1. Daily maximum and minimum air temperature at 20 cm (˚C), precipitation (mm, date is that of collection following cessation) and $\delta^{18}O$ of precipitation (‰) during the 2004-growing season.

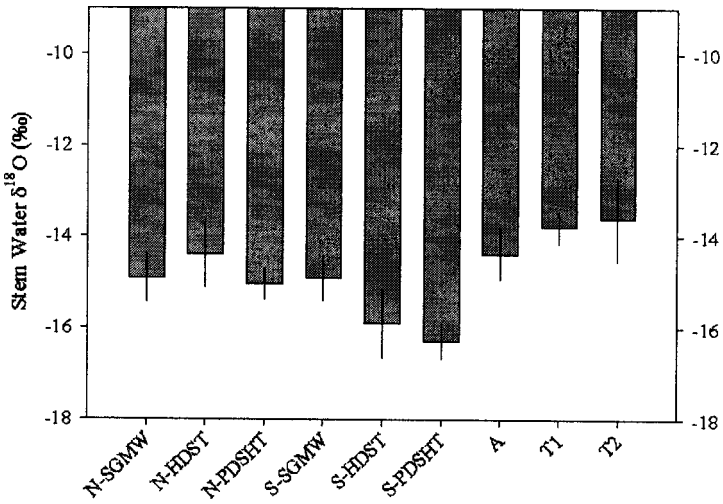

Figure 4.2. Stem water $\delta^{18}O$ (‰) in each zone or treatment at the three study sites over three sampling dates in July 2004. Bars are 1.0 SE.

$\Delta^{18}O$ vs. g_s

Simple linear regression of cellulose $\delta^{18}O$, which does not account for variation in source water $\delta^{18}O$, on cellulose $\Delta^{18}O$ revealed a weak (r^2= 0.15), but significant (P<.0001) positive correlation (Figure 4.3). Simple linear regression of the cellulose $\delta^{18}O$ residuals (observed – predicted if slope= 1) on cellulose $\Delta^{18}O$ revealed that the slope of the correlation between cellulose $\delta^{18}O$ and $\Delta^{18}O$ deviated significantly from unity (P< .0001), reflecting a correspondence between depleted source water and relatively high cellulose $\Delta^{18}O$.

Simple linear regression of leaf-level g_s on source water $\delta^{18}O$ revealed a significant positive correlation (r^2= 0.26, P= 0.0010), indicating that the highest rates of g_s were coincident with source water that was relatively enriched in ^{18}O (Figure 4.4). Source water $\delta^{18}O$ was, in turn, correlated with soil temperatures at 5 (r^2= 0.38, P= 0.0771) and 10 cm (r^2= 0.38, P= 0.0791), at the zone- or treatment-level (Figure 4.5). The most ^{18}O enriched source waters were observed in concert with the warmest soils. Stomatal conductance was highly correlated with soil temperatures at both 5 (r^2= 0.93, P= 0.0078) and 10 cm (r^2= 0.95, P= 0.0041), across zones and treatments in PDSHT (Figure 4.6). The highest rates of g_s were coincident with the warmest soils.

Zone or treatment mean cellulose $\Delta^{18}O$ and g_s were negatively correlated at each of the three sites (Figure 4.7). At North Mountain, there was a trend toward increasing g_s and declining cellulose $\Delta^{18}O$ with increasing radiation load. For every 0.10 mmol H_2O m^{-2} sec^{-1} increase in g_s, there was a 3.88‰ decline in cellulose $\Delta^{18}O$. Along the south-facing hill slope, the lowest rates of g_s and the highest cellulose $\Delta^{18}O$ were found in the HDST. The highest rates of g_s and the lowest cellulose $\Delta^{18}O$ were found in the SGMW,

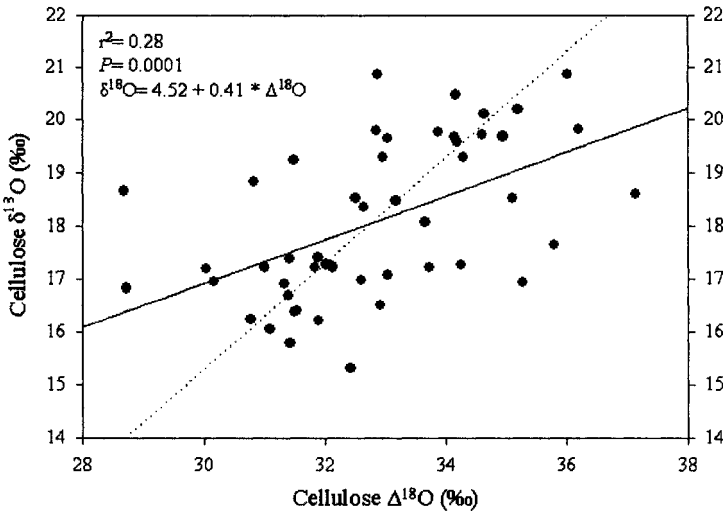

Figure 4.3. Simple linear regression of leaf cellulose $\delta^{18}O$ (‰) on leaf cellulose $\Delta^{18}O$ (‰). The dotted line, centered on median $\delta^{18}O$ and $\Delta^{18}O$, is included to show how the correlation deviates from unity.

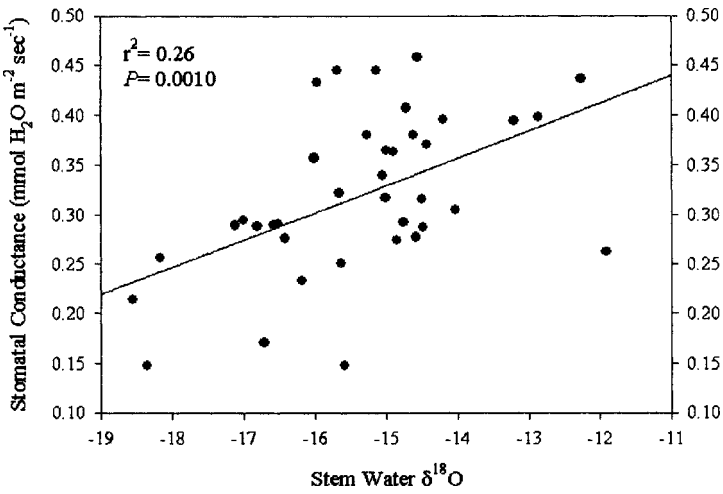

Figure 4.4. Simple linear regression of leaf-level stomatal conductance (mmol H_2O m^{-2} sec^{-1}) on corresponding stem water $\delta^{18}O$ (‰). Measurements were taken on three sampling dates during July 2004.

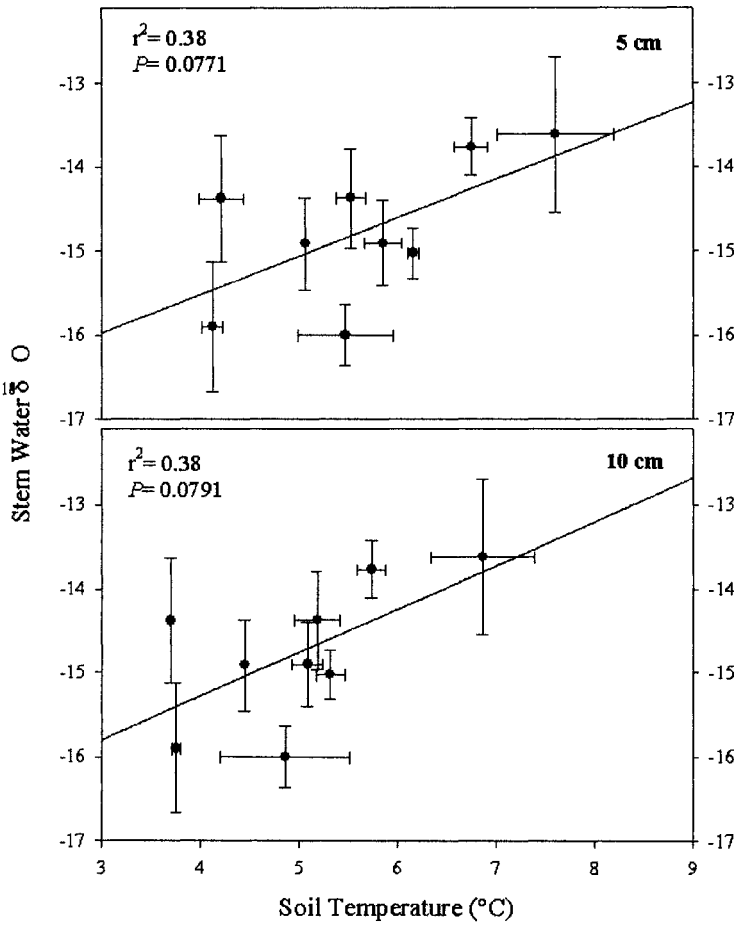

Figure 4.5. Simple linear regressions of zone or treatment mean stem water $\delta^{18}O$ (‰) on growing season mean soil temperatures at 5 and 10 cm (°C). Data were averaged over 3 sampling dates during July 2004. Bars are 1.0 SE.

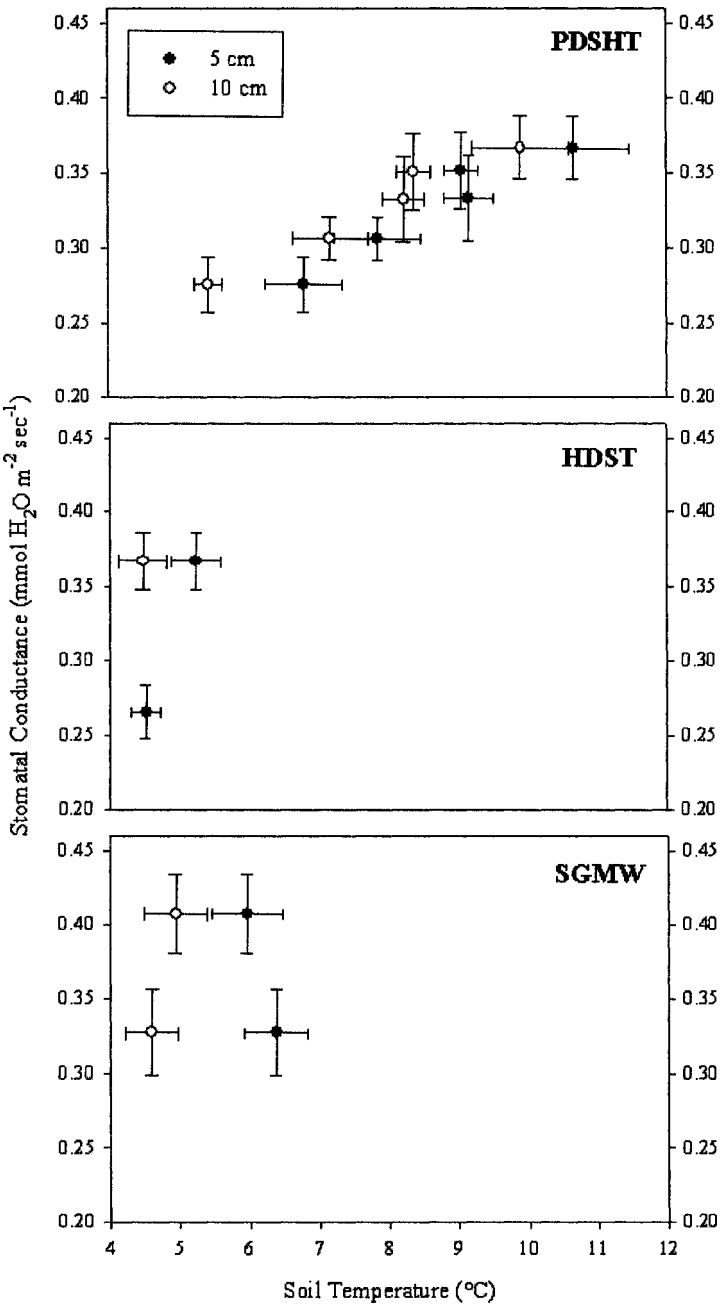

Figure 4.6. Plots of zone or treatment mean stomatal conductance (mmol H_2O m^{-2} sec^{-1}) against point measurements of soil temperatures at 5 and 10 cm (°C). Data were averaged over 3 sampling dates during July 2004. Bars are 1.0 SE.

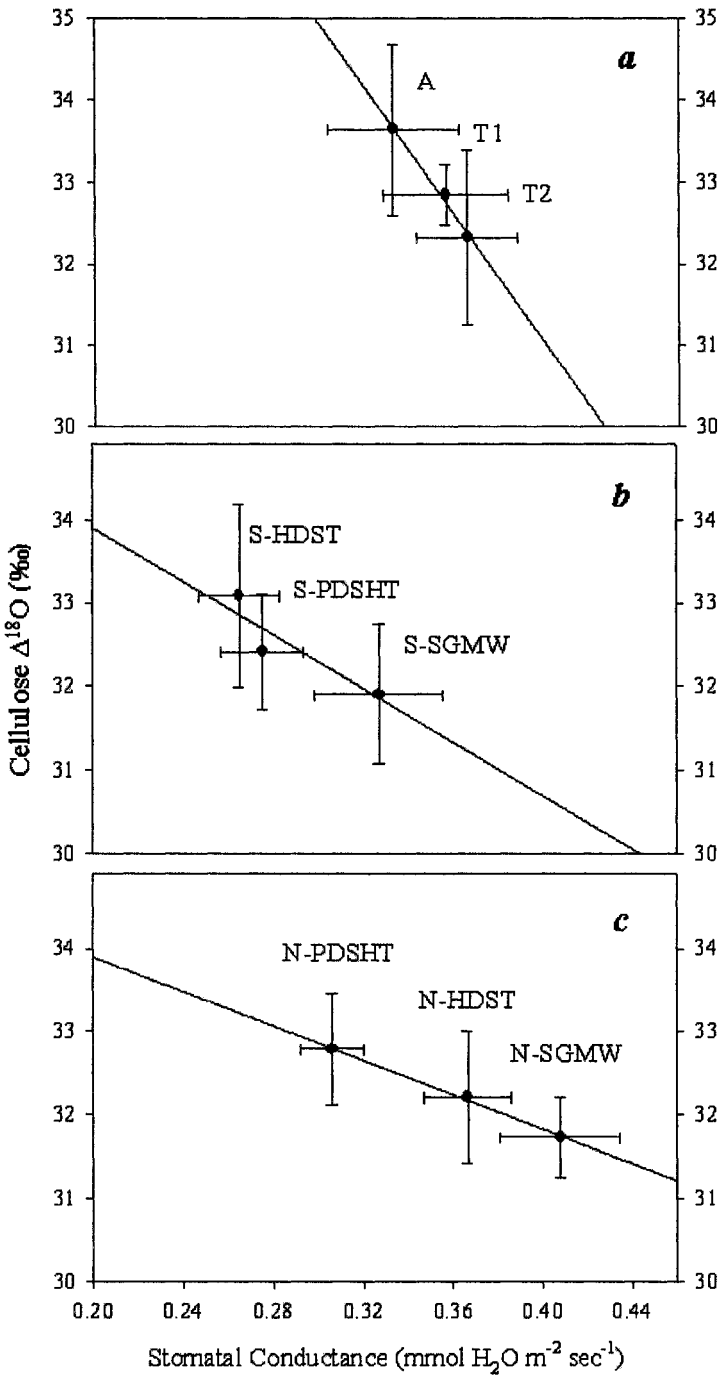

Figure 4.7. Leaf cellulose $\Delta^{18}O$ (‰) in response to variation in stomatal conductance (mmol H_2O m^{-2} sec^{-1}) in each zone of treatment at North Mountain (*a*), the south-facing hill slope (*b*) and the north-facing hill slope (*c*). Data were averaged over 3 sampling dates during July 2004. Bars are 1.0 SE.

while the PDSHT was intermediate with respect to both variables. For every 0.10 mmol H_2O m^{-2} sec^{-1} increase in g_s, there was a 1.60‰ decline in cellulose $\Delta^{18}O$. Along the north-facing hill slope, the lowest rates of g_s and the highest cellulose $\Delta^{18}O$ were found in the PDSHT. The highest rates of g_s and the lowest cellulose $\Delta^{18}O$ were found in the SGMW, while the HDST was intermediate with respect to both variables. For every 0.10 mmol H_2O m^{-2} sec^{-1} increase in g_s, there was a 1.04‰ decline in cellulose $\Delta^{18}O$.

Variability in A_{max}/g_s and Corresponding Variability in Cellulose $\Delta^{18}O/\Delta^{13}C$

Along the north-facing hill slope, there was an increase in g_s from the PDSHT to the SGMW, but relatively little change in A_{max} (Figure 4.8). The increase in g_s corresponded with a trend toward higher cellulose $\Delta^{13}C$ and lower cellulose $\Delta^{18}O$. With a 1.00‰ increase in cellulose $\Delta^{13}C$, there was a 2.00‰ decline in cellulose $\Delta^{18}O$. At North Mountain, there was a trend toward higher g_s and A_{max} with supplemental radiation. With a 0.10 mmol H_2O m^{-2} sec^{-1} increase in g_s, there was a 3.6 μmol CO_2 m^{-2} sec^{-1} increase in A_{max}. The trend toward higher g_s and A_{max} corresponded with a trend toward higher cellulose $\Delta^{13}C$ and lower cellulose $\Delta^{18}O$ with supplemental radiation. With a 1.00‰ increase in cellulose $\Delta^{13}C$, there was a 3.07‰ decline in cellulose $\Delta^{18}O$. Along the south-facing hill slope, there were increases in both g_s and A_{max} from the HDST to the SGMW. With a 0.10 mmol H_2O m^{-2} sec^{-1} increase in g_s, there was a 5.0 μmol CO_2 m^{-2} sec^{-1} increase in A_{max}. The trend toward higher g_s and A_{max} corresponded with a trend toward higher cellulose $\Delta^{13}C$ and lower cellulose $\Delta^{18}O$. With a 1.00‰ increase in cellulose $\Delta^{13}C$, there was a 5.51‰ decline in cellulose $\Delta^{18}O$.

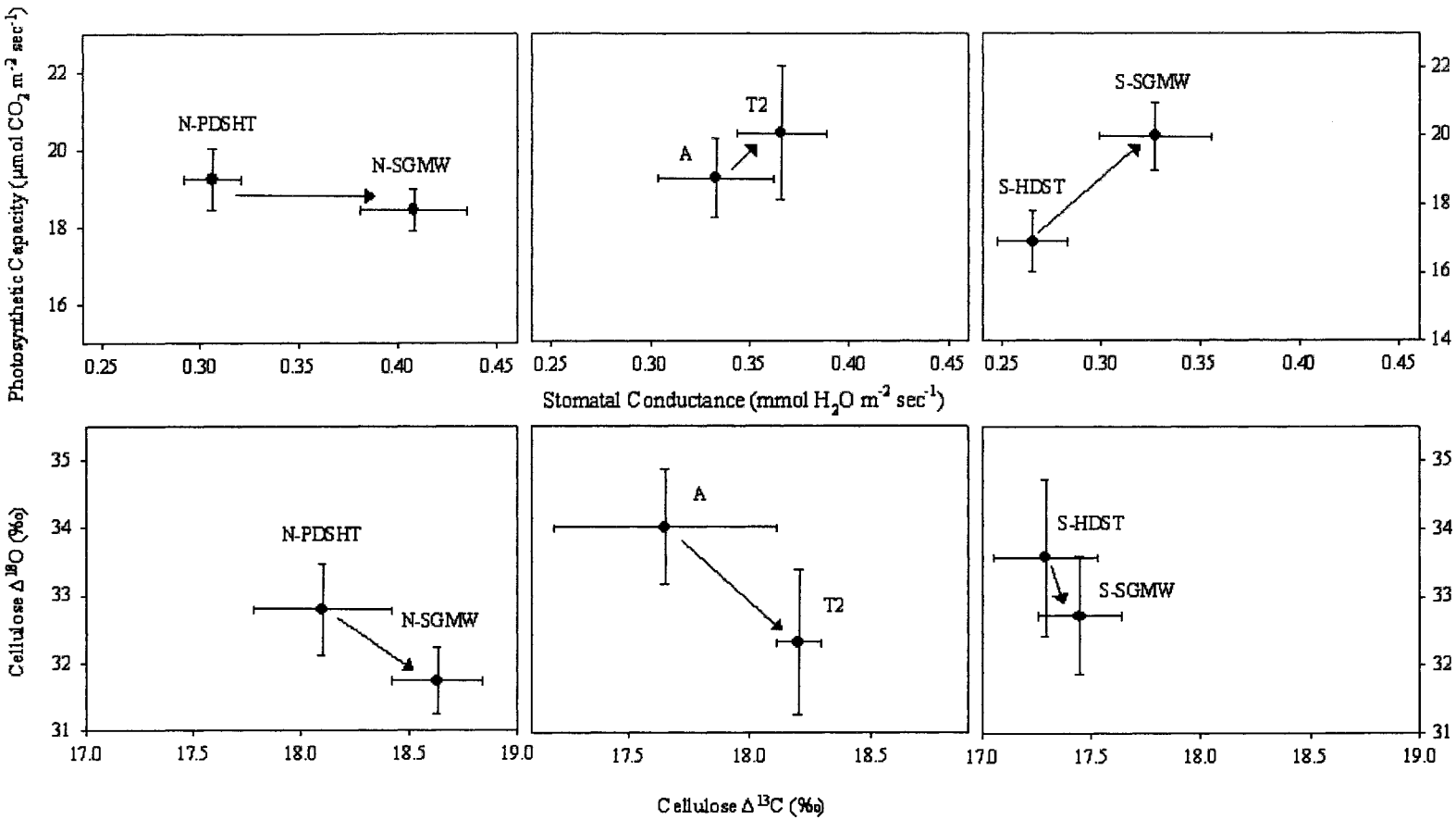

Figure 4.8. Patterns of variation in photosynthetic capacity (μmol CO_2 m^{-2} sec^{-1}) and stomatal conductance (mmol H_2O m^{-2} sec^{-1}), along with corresponding patterns of variation in leaf cellulose $\Delta^{18}O$ (‰) and $\Delta^{13}C$ (‰). Data were averaged over 3 sampling dates during July 2004. Bars are 1.0 SE.

Discussion

Oxygen Isotope Ratios in Precipitation, Stem and Leaf Waters

There was a difference of 8.8‰ between $\delta^{18}O$ of snow cores collected in early April 2004 and amount-weighted precipitation collected throughout the 2004-growing season. This difference is approximately 2.5‰ less than that observed at Toolik Lake, AK, a more continental low arctic site (Sullivan and Welker in review). The $\delta^{18}O$ of winter precipitation was similar between the two arctic sites, but summer rain was approximately 3.0‰ more depleted in Pituffik. Differences in the seasonality of precipitation $\delta^{18}O$ probably reflect the more maritime environment in Pituffik, where winters are warm and summers are cool, relative to more continental high arctic sites.

Stem water was enriched relative to both winter snow and summer rain, with a range of 2.7‰ across zones and treatments at the three sites. Enrichment of stem water above winter snow and summer rain may reflect near surface soil water evaporative enrichment, or the importance of fog as a water source for plants (Dawson 1998) in the maritime High Arctic. Fog water was highly enriched relative to rain and snow water, with a $\delta^{18}O$ signature of –7.7‰, over three collection dates during the 2004-growing season. The susceptibility of plant water sources to evaporative enrichment is supported by the observed trend toward increasing stem water enrichment with increasing supplemental radiation at North Mountain. It is noteworthy that a trend toward source water enrichment was observed in the absence of a drying effect beneath the infrared lamps. This discrepancy suggests that $\delta^{18}O$ of soil water is a more sensitive indicator of evaporative conditions than soil water content. The SGMW sites at the base of both hill slopes have a winter snow pack that is much deeper than any of the other treatments or

zones discussed in this study. Previous studies have revealed that snowmelt water can be an important water source for arctic plants (Welker et al. 1995, in press, Sullivan and Welker in review). I was, therefore, surprised to find stem water $\delta^{18}O$ values that were not different than upland sites at the N-SGMW and that tended to be more enriched than upland sites at the S-SGMW. While the SGMW sites have standing water for much of the growing season, the hummocks extend above the water table by as much as 30 cm and those at the S-SGMW tend to be taller than the hummocks at N-SGMW. *S. arctica* colonizes the tops and, to a limited extent, the sides of the hummocks, which are composed of low density structured organic matter. Source water in the raised hummocks may have been more subject to evaporative enrichment than source water at more upland sites, with hummock height and aspect contributing to the magnitude of enrichment. This hypothesis is supported by my observation that hummock soil temperatures in the S-SGMW were warmer than those in the drier, upland S-PDSHT.

$\Delta^{18}O$ vs. g_s

Barbour et al. (2004) discussed the value of expressing the $\delta^{18}O$ of leaf organic material as enrichment above source water $\delta^{18}O$ ($\Delta^{18}O$), as a method to isolate the two sources of variation in $\delta^{18}O$ of leaf organic material. Simple linear regression of cellulose $\delta^{18}O$ on cellulose $\Delta^{18}O$ revealed a weak positive correlation. The failure of cellulose $\delta^{18}O$ to account for a substantial proportion of the variation in cellulose $\Delta^{18}O$ suggests that source water $\delta^{18}O$ and LWE often vary independently. Studies that aim to differentiate between source water and LWE effects on $\delta^{18}O$ of leaf organic material should, therefore, measure source (stem) water $\delta^{18}O$ directly, unless one source of

variation can be logically eliminated (e.g., Scheidegger et al. 2000, Sullivan and Welker in review).

The correlation between leaf cellulose $\delta^{18}O$ and $\Delta^{18}O$ deviated significantly from unity, reflecting a correspondence between ^{18}O depleted source waters and relatively high leaf cellulose $\Delta^{18}O$. Isotope theory predicts that low rates of g_s may lead to high leaf cellulose $\Delta^{18}O$. The plot of g_s on source water $\delta^{18}O$ confirms that low rates of g_s were coincident with ^{18}O depleted source waters. The correlation between g_s and source water $\delta^{18}O$ is certainly not a causal relationship, as there is no mechanism by which source water isotopic composition itself could influence g_s. Source water $\delta^{18}O$ was, however, correlated with soil temperature and soil temperature was correlated with g_s in PDSHT, where I had the greatest range of soil temperatures. The correlation between g_s and soil temperature is consistent with previous work on *S. arctica* (Dawson and Bliss 1989) and other arctic plant species (Starr et al. 2004). I found that high rates of g_s were coincident with ^{18}O enriched source waters, while previous studies in the Arctic have shown relatively high rates of g_s coincident with greater use of ^{18}O depleted snow melt water (Welker et al. 1995, in press, Sullivan and Welker in review). Comparison of snow depth (Table 4.1) and source water $\delta^{18}O$ data (Figure 4.2) suggests that variation in source water $\delta^{18}O$ was driven by differential source water enrichment, rather than by differential snow melt water inputs. Therefore, across the zones and treatments I examined, source water $\delta^{18}O$ was not a faithful indicator of snowmelt water inputs.

At a given humidity, oxygen isotope theory predicts that plants with higher rates of g_s are expected to have lower LWE and lower leaf $\Delta^{18}O$, as high rates of g_s reduce leaf temperature and intercellular water vapor pressure (Craig and Gordon 1965, Dongmann

et al. 1974, Flanagan et al. 1991, Farquhar and Lloyd 1993). Studies have found that bulk LWE is frequently less than predicted for the sites of evaporation and that the magnitude of divergence increases with increasing rates of transpiration (e.g., Yakir et al. 1989). Farquhar and Lloyd (1993) suggested reduced LWE under high rates of transpiration may reflect the balance between the convective flux of unfractionated water to, and the back diffusion of enriched water from, the sites of evaporation (i.e., a Péclet effect). Under high rates of transpiration, convection is presumed to dominate and less enriched water is expected to diffuse back into the leaf. The Péclet effect, therefore, reinforces the reduction of LWE and leaf $\Delta^{18}O$ under high rates of g_s.

Cellulose $\Delta^{18}O$ and g_s were negatively correlated across zones or treatments at each of the three sites, consistent with theory and results in greenhouse (Barbour and Farquhar 2000) and agricultural settings (Barbour et al. 2000). I found the steepest slope in the correlation between cellulose $\Delta^{18}O$ and g_s at North Mountain, where there was a trend toward higher g_s and lower cellulose $\Delta^{18}O$ in response to supplemental radiation. I found an intermediate slope at the south-facing hill slope. The highest rates of g_s and the lowest cellulose $\Delta^{18}O$ occurred in concert with relatively high soil water contents and warm soils in the S-SGMW, while the lowest rates of g_s and the highest cellulose $\Delta^{18}O$ occurred along with relatively high soil water contents and low soil temperatures in the S-HDST. The S-HDST was the only zone or treatment where I found significantly lower stem water potentials (Table 4.2). Reduced rates of g_s and relatively low stem water potentials in the S-HDST were probably a consequence of low soil temperatures. I found a relatively shallow slope in the correlation between cellulose $\Delta^{18}O$ and g_s at the north-facing hill slope, where variation in cellulose $\Delta^{18}O$ and g_s generally tracked with

Table 4.2. Variation in gas exchange physiology and water relations of *Salix arctica* across three sampling dates during July 2004 in each zone or treatment at the three study sites. Standard deviations appear within parentheses. Entries marked with a different superscript are significantly different at $\alpha = 0.05$.

Site	A_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	g_s ($mmol\ H_2O\ m^{-2}\ s^{-1}$)	c_i/c_a	Stem WP (MPa)	Leaf N (mg/g)
N-SGMW	18.5 (1.9)^{a}	0.408 (0.092)^{a}	0.773 (0.038)^{a}	-0.58 (0.17)^{a}	37.4 (7.2)^{a}
N-HDST	21.4 (4.3)^{a}	0.367 (0.066)^{ab}	0.714 (0.057)^{ab}	-0.52 (0.10)^{a}	30.4 (4.9)^{ab}
N-PDSHT	19.3 (2.6)^{a}	0.306 (0.047)^{ab}	0.696 (0.027)^{ab}	-0.50 (0.07)^{a}	22.8 (4.7)^{c}
S-SGMW	20.0 (3.4)^{a}	0.327 (0.099)^{ab}	0.692 (0.088)^{b}	-0.62 (0.12)^{a}	31.2 (4.6)^{b}
S-HDST	16.9 (3.1)^{a}	0.265 (0.062)^{b}	0.698 (0.054)^{ab}	-0.66 (0.11)^{a}	25.6 (3.1)^{bc}
S-PDSHT	16.9 (3.3)^{a}	0.276 (0.063)^{b}	0.709 (0.056)^{ab}	-0.75 (0.16)^{b}	22.1 (2.8)^{c}
NM-A	19.2 (3.5)^{a}	0.333 (0.100)^{ab}	0.708 (0.068)^{ab}	-0.50 (0.10)^{a}	24.8 (2.6)^{bc}
NM-T1	19.2 (4.2)^{a}	0.356 (0.092)^{ab}	0.725 (0.070)^{ab}	-0.59 (0.13)^{a}	23.5 (3.2)^{c}
NM-T2	20.4 (5.7)^{a}	0.366 (0.075)^{ab}	0.722 (0.056)^{ab}	-0.59 (0.20)^{a}	22.6 (4.4)^{c}

variation in soil water content: SGMW > HDST > PDSHT. Across sites, the slope of the correlation between cellulose $\Delta^{18}O$ and g_s varied roughly with the atmospheric vapor pressure deficit (VPD). The hypothesis that VPD may interact with g_s to control variation in leaf cellulose $\Delta^{18}O$ will require further testing.

Variability in A_{max}/g_s and Corresponding Variability in Cellulose $\Delta^{18}O$/ $\Delta^{13}C$

Theory predicts that when variation in $\Delta^{13}C$ is driven by changes in g_s, a negative relationship between $\Delta^{13}C$ and $\Delta^{18}O$ will emerge because, for instance, rising g_s increases c_i/c_a and reduces LWE. In contrast, when variation in $\Delta^{13}C$ is driven by changes in A_{max}, there should be no relationship between $\Delta^{13}C$ and $\Delta^{18}O$, as A_{max} does not affect $\Delta^{18}O$ (Yakir and Israeli 1995). When variation in $\Delta^{13}C$ is driven by changes in both g_s and A_{max}, the change in $\Delta^{18}O$ for each unit change in $\Delta^{13}C$ should increase, as changes in A_{max} typically counteract effects of g_s on c_i/c_a (Barbour et al. 2002).

At the north-facing hill slope, there was an increase in g_s from the PDSHT to the SGMW, but no evidence of a change in A_{max}. The increase in g_s corresponded with a trend toward higher cellulose $\Delta^{13}C$ and lower cellulose $\Delta^{18}O$, in accord with dual isotope theory and the results of previous studies (Sternberg et al. 1989, Saurer et al. 1997, Barbour et al. 2000, 2002, Barbour and Farquhar 2000, Cernusak et al. 2005). At North Mountain, there was a trend toward higher g_s and A_{max} with supplemental radiation. The trend toward higher g_s and A_{max} corresponded with a trend toward higher cellulose $\Delta^{13}C$ and lower cellulose $\Delta^{18}O$ and, consistent with expectations, the slope of the $\Delta^{18}O$: $\Delta^{13}C$ correlation was steeper than that observed at the north-facing hill slope, where variation in $\Delta^{13}C$ was apparently driven by changes in g_s alone. At the south-facing hill slope there was an increase in both g_s and A_{max}, but the slope of the positive correlation between g_s

and A_{max} was greater than that observed at North Mountain. A steeper slope in the correlation between g_s and A_{max} corresponded with a $\Delta^{18}O$: $\Delta^{13}C$ correlation that was steeper than observed at North Mountain, suggesting that further increases in the A_{max}: g_s slope lead to a further reduction in the $\Delta^{18}O$: $\Delta^{13}C$ slope.

Conclusions

The results of my study demonstrate the dual isotope ($\Delta^{18}O$ and $\Delta^{13}C$) approach is capable of revealing patterns of variability in *S. arctica* leaf physiology throughout the High Arctic landscape and in response to experimental climate change. Stem water $\delta^{18}O$ and LWE often varied independently. Consequently, leaf cellulose $\delta^{18}O$ would not have been a faithful indicator of variability in g_s. Stomatal conductance was highly correlated with soil temperature across zones and treatments in PDSHT. Cellulose $\Delta^{18}O$ was consistently negatively correlated with g_s, though the slope of correlation varied across sites, in rough accordance with variation in the VPD. Cellulose $\Delta^{18}O$ and $\Delta^{13}C$ were also consistently negatively correlated. The slope of the $\Delta^{18}O$: $\Delta^{13}C$ correlation was a function of the relative importance of A_{max} in driving variation in $\Delta^{13}C$. When A_{max} was invariant, the slope of the $\Delta^{18}O$: $\Delta^{13}C$ correlation was relatively shallow. When A_{max} increased in concert with rising g_s, the slope of the $\Delta^{18}O$: $\Delta^{13}C$ correlation steepened and became progressively steeper with an increase in the rise in A_{max} per unit rise in g_s. Thus, variation in the slope of the $\Delta^{18}O$: $\Delta^{13}C$ correlation allowed for qualitative assessment of the relative importance of A_{max} and g_s in determining leaf cellulose $\Delta^{13}C$. The frequency with which my results were qualitatively consistent with isotope theory suggests a more quantitative dual isotope model may be a realistic goal.

CONCLUSIONS

Open-top chamber (OTC) warming in tussock tundra affected air and soil surface temperatures, but did not affect temperatures beneath the soil surface. Snow fences increased the depth of the winter snow pack by 0.5-1.0 m in the intermediate zones, increased winter soil temperatures, affected rates of net nitrogen mineralization, shortened the growing season by approximately 3 weeks and increased early-season soil water contents in tussock and dry heath tundra. Overhead infrared lamps warmed the vegetation canopy and soils to depths greater than 10 cm, but did not affect soil water contents in prostrate dwarf-shrub, herb tundra. In general, infrared lamps increased temperatures in the vegetation microclimate with fewer artifacts than OTC warming. OTC's did, however, provide an opportunity to examine the connectivity of abiotic changes above- and biotic responses belowground, as they did not warm the soils of tussock tundra.

Vegetation physiology was affected by experimental climate change. Changes in physiology were apparent in the response of *Eriophorum vaginatum* to OTC warming near Toolik Lake, the responses of *Ledum palustre* and *Vaccinium vitis-idaea* to deeper snow near Toolik Lake and the response of *Salix arctica* to supplemental radiation near Pituffik.

Eriophorum vaginatum increased rates of early season leaf and root growth within the chambers, but growth rates declined to ambient levels by mid-season. There was no evidence that OTC warming increased annual *Eriophorum vaginatum* production, above- or belowground, despite higher rates of early season growth. This observation is

consistent with the hypothesis that carbon supply limits the rate of arrival at peak biomass, while nutrients limit the magnitude of annual production.

Leaf gas exchange physiology of *Ledum palustre* and *Vaccinium vitis-idaea* responded to deeper snow, but responses differed for the two species and varied across ecosystems. Leaf oxygen isotope and nitrogen analyses revealed qualitatively similar effects of deeper snow on the two species, but the effects differed across ecosystems. Leaf carbon isotope analyses revealed that changes in snowmelt water inputs and leaf nutrient concentrations, which differed across ecosystems, had divergent effects on the gas exchange physiology of the two evergreen species.

Leaf gas exchange physiology of *Salix arctica* varied across landscape gradients and in response to supplemental radiation. In some instances, photosynthetic capacity and stomatal conductance varied in tandem, while in other cases, variation in stomatal conductance was not attended by variation in photosynthetic capacity. Patterns of variation in the ratio of intercellular to ambient partial pressures of CO_2 were faithfully recorded in the carbon isotope ratios of leaf cellulose. Patterns of variation in stomatal conductance were faithfully recorded in the oxygen isotope ratios of leaf cellulose, when variation in source water was removed. Changes in carbon isotope ratios attributable to changes in photosynthetic capacity and those attributable to changes in stomatal conductance were successfully identified when the information contained in carbon and oxygen isotope ratios was combined.

These studies demonstrate that arctic plant physiology can be highly sensitive to increases in air temperature, canopy and soil temperatures and or soil water contents. The physiological responses observed were not, however, simple. The manipulated

variables often acted indirectly, through other variables, and responses in one part of the plant were often mediated by responses in another part of the plant. Species of the same functional group showed divergent responses to the same driving variables and responses of a single species were dependent upon ecosystem type.

Stable isotopes of carbon and oxygen have been used infrequently in studies that examine ecosystem responses to experimental climate change. Testing the dual isotope approach with gas exchange measurements revealed that combined use of carbon and oxygen isotopes is a powerful method to describe variation in leaf gas exchange physiology across landscape gradients and in response to experimental climate change.

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